II. *Observations on the Development of Ornithorhynchus.*

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Introduction.

(1) *Collection of Material.*

The records of Australian biology bear sufficient witness to the fact that it is extremely difficult, even for residents in Australia, to procure the material necessary for an investigation into the development of the egg of Ornithorhynchus. It is only through the organisation of special expeditions, with ample resources both of time and money, that any large measure of success in this direction can be hoped for.

The animal itself, though pretty widely distributed, and probably still far from becoming extinct, is to be found, in any one locality, only in comparatively small numbers. It is now much less plentiful than formerly, owing to the demand for its fur—a demand which is still satisfied in spite of the measure of legal protection which the animal has obtained in the various Australian States. The depredations of the fur-hunter are not easily repaired, since the animal breeds only once in the year and produces but two eggs at a time.

The eggs, when laid, are deposited in a burrow which it is far from easy to locate, and whose opening up involves a considerable amount of labour, since, apart from its great length, the river-bank in which it is situated is commonly enough permeated by tree-roots. And when at length the actual dwelling-chamber or nest is successfully opened up, no reward at all may be forthcoming, or the material which is obtained may be unsuitable for the immediate purpose in view.

Even when it is the intra-uterine stages of the egg which are required—as was chiefly the case in connection with the present research—the difficulties are nearly as great. The animal is extremely shy and difficult of approach. They are occasionally, but rarely, captured as an incident in net-fishing in the larger rivers: otherwise they are practically only obtainable with the gun. During the breeding season, however,

the pregnant female appears to keep much more closely to the burrow, so that one may then commonly enough shoot five or six males to one female.

Again, the breeding period appears to be subject to some variation, both as regards locality and character of season. We have only to a very limited extent been able to superintend personally the work of collection, and have found it necessary to rely largely upon the efforts of the scientifically untrained collector in the accumulation of the material for our investigations. Unfortunately, the material result of the work of such a collector during an entire season is so meagre that he requires considerable inducement to carry on the work at all; whilst the product to the investigator may be practically nil. The genuineness of the difficulty in procuring this kind of material is sufficiently indicated by the fact that whilst SEMON ('94), in his expedition, was successful in obtaining a very considerable number of eggs and fœtal specimens of Echidna, his collection of Ornithorhynchus embryos would appear to have been limited to a small number of early intra-uterine eggs.

Our own collection is very far indeed from being complete, though the work of collection has been going on for quite a number of years.

The expenses of one of us (H.) have been in part defrayed by grants from the Government Grant Committee of the Royal Society of London, and the work was in part carried out during his tenure of a "George Heriot" Research Fellowship in Zoology, during the years 1899 and 1900.

We desire to express our indebtedness to the Trustees of the Australian Museum, Sydney, for our acquisition, through the courtesy of Mr. R. ETHERIDGE, the Curator, of one of the specimens described in this paper; also to Mr. JOHN JOHN for valuable voluntary assistance from time to time in the work of collection.

(2) *Technique.*

Material for investigation in the shape of intra-uterine eggs of Ornithorhynchus is not by any means easy to carry successfully through all the various stages of technical manipulation. This statement is, however, chiefly applicable to the middle period of uterine sojourn with which we are mainly concerned in the present paper. It is relatively easy to treat the earliest and the latest stages of the uterine egg. In the former the yolk is a tolerably compact mass which fixes in solid form. In the latter the embryo and its membranes have assumed a definite and coherent condition. The difficulties attending the treatment of the intermediate condition, represented by the cellular wall of a blastodermic vesicle with fluid contents, will later on be adverted to. Fixation of the egg *in toto* is easily enough carried out, even at the hands of a non-expert collector; but the entire subsequent procedure is fraught with danger to the specimen.

The technical methods employed by us embrace no very novel procedures in the way of departure from methods ordinarily adopted.

KLEINENBERG'S picro-sulphuric or picro-nitric fluids were employed as the usual

fixative in the case of the specimens collected in the earlier years. Latterly, picro-corrosive-acetic fluid has been generally used.

For several years we have with much advantage followed a modification of JORDAN'S method of double-embedding in cedar-oil-celloidin and paraffin. We now regard double-embedding as indispensable for all embryological work of a critical character. It is only necessary to contrast serial sections of chick blastoderms prepared by this method with those obtained after embedding in paraffin alone, to become convinced of the inability of the latter method to do complete justice to the details of the structure and relations of the embryonic tissues.

Sections have been mounted on slides treated with MAYER'S albumen and flattened out on water on the top of the paraffin-bath. We have found it advisable, in the case of double-embedded tissues, to coat each slide, after the sections have thoroughly dried on, with a thin solution (0·5—0·75 per cent.) of celloidin. Unless this is done, their complete adhesion throughout the subsequent staining and mounting processes cannot be so completely relied upon as in the case of simple paraffin-embedded sections. As soon as the thin film of celloidin has set, the slides may be placed at once in a jar of 90-per-cent. alcohol to which 10 per cent. of chloroform has been added. They may then be treated like ordinary serial slides, save for the proviso that, wherever alcohol of 90 per cent. or over is utilised, it should contain an addition of 10 per cent. of chloroform. The processes of staining are not interfered with, through the above procedure, otherwise than by a very slight slowing.

Sections have, as a rule, been stained with hæmatoxylin or hæmatein, and counter-stained with eosin. Sometimes iron-hæmatein has been used, more often dilute DELAFIELD'S hæmatoxylin or an equivalent solution. In one or two cases borax-carmine was used, and for certain purposes picric acid was employed as a counter-stain to the hæmatein.

In surface-observation we have derived great advantage from the use of the Braus-Drüner binocular stereoscopic microscope. It is hardly too much to say that the use of an instrument of this character adds a new value to the low-power observation of an object, *in toto*. In a few cases it has been possible to utilise the resources of the same instrument for stereoscopic photography; but when used in camera form, and necessarily without eye-piece, its magnifying power is very limited.

(3) *Illustration.*

We have made extensive use of the resources of photography, both for the purpose of graphically recording the progressive results of our work, and also, as the plates accompanying this memoir show, of final illustration. We are not unconscious of the fact that in so doing we sacrifice the opportunity, which the ordinary drawing affords, of rendering certain points diagrammatically clear. The drawings that we have thought it advisable to reproduce as plates will show that our decision to trust mainly to photographic reproduction was in no way forced upon us. But not a few

of the facts recorded in the following pages seem to us of such critical interest as to demand the utmost degree of objectivity in their presentation. For this we have been content to rely upon the modern methods of photographic reproduction from our untouched photographs.

Æsthetically considered, no doubt, photographs, with their frequently dingy or spotty backgrounds, and variegated with out-of-focus patches, may seem vastly inferior to the careful and finished drawing. From the scientific point of view, on the other hand, it is difficult to see what can be urged against the photograph when questions of evidence are involved, provided that it definitely affords the evidence it purports to supply. It is certainly devoid of the subjective element which pervades even a *camera-lucida* drawing. For merely didactic purposes, of course, a totally different criterion is applicable. But in records of original investigation, in which the structural appearances to be represented are obscure, and their significance equivocal, a photograph is tantamount to an original document to which appeal may be made with something of the same confidence as to the object itself.

In connection with this matter of photography we desire to acknowledge the unwearied assistance we have received in the preparation of our photographs from Mr. Louis Schæffer, Laboratory-Assistant in the Anatomy Department of the University of Sydney.

(4) *Literature.*

We have avoided the appearance of an attempt to provide an exhaustive account of the scientific literature of the several subjects dealt with. The list of papers appended consists merely of those to which reference is actually made in the body of the paper, and does not include those merely consulted in connection with the work. Further bibliographical information is readily obtainable by the consultation of the lists appended to various of the works quoted, especially the appropriate chapters in Hertwig's 'Handbuch,' and Keibel's review of present-day work on gastrulation, in Merkel and Bonnet's 'Ergebnisse' for 1900, vol. 10. A tolerably comprehensive survey of the literature bearing on the subjects of neuromeric segmentation and early ganglionic development will also be found in connection with the work of Neal ('98), quoted below. Neal's list is brought up to date by the references given in the quite recent paper of J. B. Johnston ('05).

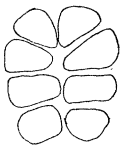
Chapter I.—DESCRIPTIVE ACCOUNT OF A NUMBER OF STAGES IN THE DEVELOPMENT OF ORNITHORHYNCHUS.

(1) Early Segmentation Stage. (Specimens A and AA.)

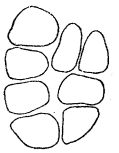
The earliest stage of the egg of Ornithorhynchus with which we have worked is represented by two twin eggs, distinguished under the letters A and AA in our collection. The eggs measured 5·5 millims. in diameter.

A photograph of the Specimen A, under a magnification of 10 diameters, is reproduced in fig. 1, Plate 1.

Both of the eggs were in the eight-celled segmentation stage, and the eight segments or blastomeres in Specimen A may be faintly distinguished in the photograph. In both cases a definite bilateral symmetry is apparent. Text-figs. 1 and 2 illustrate the form and arrangement of the blastomeres in the two specimens. It will be observed that in Specimen AA the symmetry is imperfect, owing to an irregularity in the position of two of the cells on one side.



TEXT-FIG. 1.



TEXT-FIG. 2.

Text-fig. 1 (Spec. A) and Text-fig. 2 (Spec. AA).—Outline Figures showing the Arrangement of the Blastomeres in two twin eggs belonging to the eight-celled segmentation-stage. Magnif. = about × 30.

SEMON ('94) has described and figured a surface view of a stage of an Echidna egg (his E_1, Taf. viii, figs. 1 and 10) showing four segmentation cells. These are approximately equal in size. CALDWELL ('87) had previously stated that the first segmentation gave rise to two unequal segments. The occurrence of such a primarily unequal segmentation is not borne out by the appearance of SEMON'S four-celled stage. And if, as is most probable, the primary furrow corresponds to the median line of our bilaterally symmetrical eight-celled stage, the first segmentation cannot have been unequal.

CALDWELL, in his fig. 3, Plate 31, shows a section through an egg of 2·6 millims. in diameter taken from the open end of the oviduct. It is stated to have been in the stage of eight segmentation-nuclei. Two cell-areas are shown in the figure, which is very similar to SEMON'S sectional figure of his early stage ('94, Taf. ix, fig. 30).

(2) LATER SEGMENTATION STAGE. (SPECIMEN NN.)

Our next stage is one showing a later phase of the segmentation process. It was an intra-uterine egg of 5 millims. in diameter, and appears in our list under the reference letters NN. It was fixed in picrosulphuric fluid, stained *in toto* in picrocarmine and cut in paraffin.

The stage is intermediate between SEMON'S Specimen E_2 and his O_1 (*cf.* his Taf. ix, figs. 31 and 34); and it probably also corresponds to that of CALDWELL'S fig. 4, Plate 31, also from a 5-millim. intra-uterine egg of Echidna.

The segmenting blastoderm lies upon the bed of white yolk, which is continuous with the white-yolk centre of the entire yolk-mass. The white-yolk-bed is surrounded by the large-sphered material of the yellow yolk, as indicated in fig. 2, Plate 2. Fig. 3, Plate 4, is a photograph of one of the vertical sections of this blastoderm not far from its periphery, in which plane only four of the segmentation-cells are apparent. These are partially separated superficially by surface cleavage-furrows. Each blastomere consists of a central well-defined kernel of finely granular cytoplasm surrounding the nucleus, and a peripheral zone of coarsely granular, deutoplasmic character, almost identical in its visible characters with the underlying mass of unsegmented white yolk. The deutoplasmic zones of the several cells, whilst clearly separated from one another superficially by the cleavage-furrows, are, in their deeper portions, completely continuous with, and indistinguishable from, one another. Their material is only slightly differentiated from the underlying white yolk.

The drawing reproduced in fig. 2, Plate 2, represents a section through the central part of the cellular blastoderm. Here over a dozen cells are met with. The majority of these are superficially placed and similar to those in the photograph reproduced in fig. 3, Plate 4. Four of them, however, are deeply placed, and there is, as yet, no trace of segmentation in the coarsely granular deutoplasmic material surrounding them; although their clear central, nucleated, cytoplasmic masses are quite definitely differentiated.

If comparison be made between our illustrations and SEMON'S figs. 31 and 34, Taf. ix, and also with CALDWELL'S fig. 4, Plate 31, it will be apparent that in none of the latter is there to be recognised the differentiation we have shown between deutoplasmic and cytoplasmic zones in the early segmentation-cells. Its objective character is clearly demonstrated in the photograph in fig. 3, Plate 4.

But other sections from the same specimen tend to show that the cytoplasmic kernel of the segmentation-cell grows at the expense of the peripheral zone of deutoplasmic material, so that by and by the latter disappears. So long as the peripheral zone of deutoplasm is present, the margin of the enclosed cytoplasmic mass has a ragged outline. On the disappearance of the deutoplasmic zone, the periphery of the cell is seen to be formed by the sharp contour of a purely cytoplasmic body.

(3) EARLY STAGE OF GERMINAL-LAYER FORMATION. (SPECIMEN O.)

The next stage at our disposal was represented by an intra-uterine egg, which measured 4 millims. in diameter after removal of the shell. This egg had, like the last, been fixed in picrosulphuric fluid, and was stained in bulk in picrocarmine. It is distinguished in our list as Specimen O. It belongs to the period of the commencing formation of the germinal layers. Its sectional characters reveal an essential correspondence with SEMON'S Specimen E_5.

The cellular blastoderm is here almost exclusively arranged in the form of a much attenuated epithelial membrane closely applied to the deep surface of the relatively thick vitelline membrane, and covering part of the yolk-mass, exactly as illustrated in SEMON'S figs. 22, Taf. viii, and 33, Taf. ix, from his Specimen E_5, which our specimen in all probability closely resembled.

But there are otherwise some marked differences from the condition illustrated by SEMON. There was absolutely no trace, in our complete series of sections through the embryonic pole, with its white-yolk-bed, of any nuclei whatsoever occupying the deep position in which a group of nuclei is represented by SEMON. On the other hand, the blastoderm in our specimen shows small patches in which the cells are not flattened and attenuated, but plump and oval or angular. Here and there, though rarely, these slightly overlap one another. In a few sections, indeed, one or two plump cells become isolated and detached from the otherwise single layer, though still lying close underneath it. This condition is illustrated in fig. 4, Plate 2. It is difficult to say whether these cells are naturally detached from the single layer or not. If they are, then one of two possible interpretations may be assigned to them: (*a*) They may be the last remaining deeply-placed members of the group of segmentation-cells found at the close of the previous stage, which have not yet become intercalated into the one-layered membrane which SEMON believes to be the condition attained by the blastoderm in eggs of about this same period of development (*cf.* his O_2 and O_3); or (*b*) they may represent the very earliest product of that formative activity of the embryonic region of the blastodermic membrane which issues later in the establishment both of "cenogenetic" yolk-entoderm, and of the primitive knot, to be described later on in this paper.

In any case, whilst the entire peripheral region of the blastoderm, and parts of the central area, present a condition of the membrane precisely similar to that shown in SEMON'S fig. 33, Taf. ix, patches of the central (embryonic) region present rather the appearance which seems to have characterised the entire embryonic area of the blastoderm in SEMON'S O_3, as shown in his fig. 38, Taf. ix.

(4) STAGE OF BILAMINAR BLASTODERMIC VESICLE, OR SO-CALLED "FIRST PHASE OF GASTRULATION." (SPECIMEN "ALPHA.")

In the next stage available for our study a very important advance has been made in the organisation of the blastoderm. This stage is represented in our collection by an egg distinguished as "alpha" in our catalogue of material. This egg measured 6·5 by 6 millims. in diameter. In consequence of this increase in size, the yolk-mass no longer occupies so entirely the interior of the egg; and partial disintegration of it as a solid mass has begun.

The most conspicuous advance has occurred in connection with the cellular blastoderm. This has now extended around the whole interior of the egg, and

it is already bilaminar throughout, owing to the establishment of a complete lining of yolk-entoderm surrounding the partly fluid vitelline contents of the egg. Through these changes the yolk-laden ovum has become transformed into a bilaminar blastodermic vesicle. Throughout the greater part of its extent the structure of the blastodermic membrane is very simple. It consists of an extremely attenuated ectodermal cell membrane, closely adherent to the deep surface of the vitelline membrane, so that, except where accidentally detached, it is only recognisable through the spindle-shaped sectional outlines of its flattened nuclei. To the deep surface of this is applied the layer of yolk-entoderm. The cells of this layer are individually much larger in their superficial extent than those of the overlying ectoderm. The central portion of each cell is distended by the presence of a large vesicular nucleus, and of yolk-spheres of varying size. The marginal portions of the cells are thin and membranous.

That region of the wall of the vesicle which appears to represent the early embryonic region forms a patch of about 0·5 millim. in its greater diameter. As the periphery of this area is reached, the ectoderm assumes a different character to that described above. Its constituent cells are there found to be cuboidal and much more closely packed together; whilst indications of a two-layered condition appear. Under this area the yolk-entoderm extends, unchanged in character.

Fig. 5, Plate 2, shows a drawing of a section through the embryonic patch of the blastoderm nearer its centre. Here it is seen that the yolk-entoderm preserves its character as a continuous membrane. The superficial layer of cells is irregular and consists of polymorphous cells, which, towards the margins of the field, pass into the attenuated general ectoderm of the vesicle wall. Beneath the surface-layer within the embryonic patch is an accumulation of cells of irregular shapes and sizes, which appear to be actively proliferating, and also to be in proliferative continuity with the overlying surface-layer, forming, together with these, a cake of cells, lenticular in section. This cake of cells had evidently occupied the concavity which is visible on the surface of the underlying yolk-bed, beneath which the white yolk shows many vacuoles of varying size. In other sections near the centre of the area the cell-cake is still more pronounced, presenting in its centre an even sharper prominence towards the yolk.

Except as regards the complete differentiation of yolk-entoderm, the structural characters of this "embryonic region" suggest its equivalence to a "primitive plate" in WILL'S sense. We incline to the opinion that it does in some degree bear this interpretation, in spite of the important distinction referred to, the yolk-entoderm having been "precociously" differentiated.* But it is to be noted that in any case

* Reference may be made to WILL'S discussion of the origin of the entoderm in reptiles, in his paper on Lacerta ('95), pp. 5–6: "Von Wichtigkeit ist, dass auch bei den Eidechsen vor dem Auftreten der Primitivplatte zu keiner Zeit ein Stadium existirt, in dem Ectoderm und Entoderm vollständig von einander getrennt sind."

there is practically no ectodermal shield-differentiation around this primitive plate. This "primitive plate" we take to be the initial stage in the formation of the primitive knot, which will form the subject of future description.

The gap which separates the last two stages ("O" and "alpha") is an unfortunate one, since, in the absence of an intermediate stage, we are unable to demonstrate the mode of origin of the yolk-entoderm in Monotremes. But in view of the fact that, in the earlier stage O, the yolk itself appears to be absolutely non-nucleated, the yolk-entoderm cells probably owe their origin to the proliferative activity of some of the cells which in that stage were shown underlying the otherwise unilaminar blastoderm.

It has become usual to regard the establishment of a bilaminar blastoderm, through the appearance of an entodermal or second germinal layer, in the Amniota, as a partial and indirect manifestation of the process of gastrulation. Hence this establishment of a second or entodermal layer has been recognised by HUBRECHT, KEIBEL, and others as constituting a "first phase of gastrulation."

We see no reason to believe that the processes representing this "first phase" in Ornithorhynchus differ in any essential way from those which operate to like purpose in the similarly meroblastic ova of other amniotes. And we are more than doubtful whether the surface-depression referred to and figured by SEMON (*loc. cit.*) as possibly representing the commencement of an actual invagination-process, will really bear any such interpretation; inasmuch as, in the stage in question, the blastoderm was apparently still merely unilaminar.

(5) "GASTRULAR STAGE" PROPER, OR SO-CALLED "SECOND PHASE OF GASTRULATION." (SPECIMENS D, DD, Y, AND Q.)

The so-called "second phase of gastrulation" consists in the appearance of certain structural features and processes, more or less generally prevalent in the Amniota, and which, unlike the phenomena of the "first phase," are obviously to be interpreted in terms of an actual invagination process.

On the completion of this stage of development the embryo has attained the full morphological status of the gastrula. A structural condition in Ornithorhynchus, evidently requiring such an interpretation, formed the subject of a former brief communication by the present writers, published in the 'Proceedings of the Royal Society of London' ('03).

In that communication we showed that in the egg of Ornithorhynchus of 9–10 millims. in diameter there is to be found a knob-like thickening of the cellular wall of the blastodermic vesicle, constituting a very definite "primitive knot." This knot possesses a very well-marked gastrulation-cavity ("Mesoderm-säckchen" of HERTWIG) with a wide blastoporic aperture; and it is, as yet, a completely isolated and independent structure, in spite of the fact that a primitive

streak is already in existence, separated from the knot by an interval of considerable extent.

At the time of writing our preliminary paper there were at our disposal only two specimens illustrating the condition dealt with. Since that time two other eggs have come into our possession which belong to the same period of development. Our knowledge of its characteristics is therefore now based upon the examination of four eggs, which are distinguished in our catalogue as Specimens D, DD, Q, and Y respectively. Specimens D and DD were twin eggs, and it was chiefly the latter of these which formed the subject of our earlier description.

(a) *Description of Individual Specimens of "Gastrular Stage."*

Specimen DD measured 10 × 9·5 millims. in diameter. Its shell was tolerably thick (0·02 millim.) and opaque. Within it was a moderately thin "vitelline membrane" (Zona pellucida ?). Intimately applied to the inner surface of the vitelline membrane was the thin and delicate cellular blastodermic membrane, whose innermost layer is everywhere composed of yolk-entoderm. The interior of the egg was occupied by mainly fluid contents.

So long as the diameter of the intra-uterine egg in Ornithorhynchus does not exceed 5 millims., its contents consist of a more or less compact yolk-mass having a structure like that described and figured by SEMON.

The further increase in the diameter of the egg is evidently conditioned by absorption of fluid from the uterus. This process soon brings about partial liquefaction and disruption of the yolk, so that numbers of yolk-spheres become dispersed throughout the cavity of the developing egg, and many are found adherent to the deep surface of the yolk-entoderm layer. Further, from the time of its earliest recognition the cells of the latter are found more or less distended with yolk-spheres of various sizes.

It may be noted that the accumulation of fluid which ultimately occasions disintegration of the yolk-mass takes place at first around its periphery, and it therefore does not for a considerable time undergo complete disintegration into its constituent yolk-spheres. In eggs of 12–13 millims. in diameter we still find a tolerably large remainder of the original yolk as a more or less coherent mass, lying free within the cavity of the vesicle. In eggs of 6–8 millims. diameter practically the entire yolk of the 5-millim. egg remains as a spheroidal mass, adherent to the deep surface of the blastoderm (*cf.* fig. 5, Plate 2) in the embryonic region, but elsewhere surrounded peripherally by the accumulating fluid derived by absorption from the uterine environment.

On cutting the egg open and examining the deep or inner surface of the blastoderm—the outer being still adherent to the shell—there was found a small, opaque, somewhat irregularly oblong patch, which measured 0·42 by 0·49 millim.

This proved to be a tolerably typical "primitive" or gastrula-knot, in the Sauropsidan sense, as described in our former paper.

As we there stated, no other embryonic differentiation was discovered during examination of the blastoderm *in toto*, nor had we at that time any reason to suspect the existence of any such differentiation. But the subsequent examination of the serial sections showed that, at some distance from the knot, there existed an area of considerable extent over which the ectoderm was thickened; and throughout which there ran a linear primitive streak, from whose margins a mesodermal sheet already extended outwards for a considerable distance.

Specimen D was the twin egg of Specimen DD, with which it was practically identical in size, measuring 9 millims. in diameter. In it the region of the primitive knot was easily recognisable, being rendered abnormally obvious by the fact that an extrusion of yolk had taken place at the knot from the interior of the vesicle. The extruded yolk formed a small bulging beneath the vitelline membrane, and must have occurred during fixation.*

Here again, in this specimen, no further trace of embryonic differentiation was discovered during careful examination of the portions of the opened egg viewed *in toto*, in apposition with the shell, under moderate magnification. Nevertheless, as in the case of the sister-egg, the serial sections later showed that, at some distance from the primitive knot, a primitive streak traversing an area of thickened ectoderm, and a proliferating mesodermal sheet, were also present.

The surface examination of the blastoderm of both of these specimens was carried out prior to the sectioning of either, and both were examined at a time when, as already stated, we had no reason to expect special differentiation in a region distinct, and even comparatively remote, from the quite obvious gastrula-knot. We are conscious that it may be assumed, on this ground, that our original examination of the remainder of the blastoderm away from the primitive knot must have been somewhat perfunctory. We cannot admit this to have been the case. In the instances of the two specimens, belonging to this same stage, next to be described, we had the full experience of specimens D and DD to guide us. They did not, in fact, come into our hands until after the publication of our previous paper. Yet the difficulties of examination of these specimens *in toto*, with due regard to their adequate preservation for sectioning, were such that it was only with the greatest difficulty that we were able, on surface examination, to detect in one of them (Y) the

* It is worthy of note that this extrusion had occurred actually through the blastoporic opening itself, as the serial sections subsequently proved, and had been effected by an actual evagination, or extroversion, of the wall of the gastrula-cavity or archenteron, by the underlying yolk. The blastoporic lips were completely everted around the protruding mass of yolk-spheres. The yolk-mass is, indeed, seen in section to be surrounded by a definite line, almost as if a delicate cuticular lining of the archenteric cavity, or derived from the underlying yolk-entoderm, had been evaginated along with the mass of yolk and remained stretched over it as an extremely attenuated limiting membrane.

coexistence of a primitive streak with the more obvious primitive knot. In the other (Q) the presence of the primitive streak was more readily established; but the knot escaped detection, though looked for with the greatest care, until it was ultimately discovered in the serial sections of the blastoderm. It has to be borne in mind that we are dealing with an egg which, though very large for a mammal, is not of great dimensions; that the parchment-like shell is firm and nearly opaque and that its removal as a preliminary step is entirely out of the question as soon as the egg has increased in size and become a blastodermic vesicle through the absorption of fluid. No structural differentiation is at this stage ordinarily visible through the shell, even in a cleared specimen. The opening up of the egg is therefore necessarily a random performance. After opening up, the blastoderm cannot safely be removed from the vitelline membrane and shell, with which it is in extremely intimate apposition. Thus, even when the egg has been successfully divided into two along a chance plane of section, the extreme curvature of the two pieces renders it difficult to make an adequate examination of the deeply concave surface, more especially towards the cut edges of the more or less hemispherical segments. Everyone familiar with the examination of material of this nature is aware that it is by no means sufficient merely to be able to submit every part of the surface of a blastoderm to an ocular examination. Unless it be possible to carry on the examination from different aspects and under varied lighting it is quite easy entirely to miss delicate but important structural features. Such variation of the conditions of examination is by no means easy to command in the case of the small and steeply curved segment of the Ornithorhynchus egg at the stage of development now under consideration, a stage at which the knot alone, even when recognised under low power examination, offers no clue to the orientation of the embryonic axis.

Had we not had the advantage of the binocular stereoscopic microscope, in the case of the last two specimens of the stage, Q and Y, it is not improbable that the examination *in toto* would again have proved of comparatively little value.

Specimen Q was a single egg of about 9 millims. in diameter. Its general characters resembled those of the other specimens of this stage. It was opened up by chance division into two hemispheres. On examination of the deep surface of one of these hemispheres, a primitive streak was, in this case, plainly visible. This ran obliquely towards the cut edge of the segment, which intersected it very obliquely, and not far, as it subsequently proved, from its anterior end. This latter portion of the streak was next discovered at the cut edge of the other hemisphere of the divided egg. In its entirety the primitive streak measured no less than 6 millims. in length. We made very careful search for some trace of a primitive knot in the vicinity of one or other end of the streak, but without success. We, therefore, felt at the time reluctantly compelled to assume that this specimen, although otherwise similar to those formerly described (D and DD), differed from them markedly in its phase of development.

The portions of the blastoderm containing the streak and its environment were removed for sectioning, double-embedded in cedar-oil-celloidin and paraffin, and cut in series of 10 micron sections. Their examination showed that a primitive knot was in fact present at some distance from what proved to be the anterior end of the primitive streak (*cf.* text-fig. 7) just as we had previously found to be the case in Specimens D and DD. It was situated pretty close to the cut edge of the hemispherical segment in which it lay. It had escaped detection partly, no doubt, owing to the difficulty of thoroughly investigating the margin of the hemisphere, but partly also from the fact that the cellular tissue of the knot in this specimen was less permeated by yolk spherules, and was therefore less opaque, than was the case in other specimens of the stage. At all events we failed to detect the knot on surface examination, and its apparent absence nonplussed us not a little, so that its later discovery in the section-series came as a welcome relief from our perplexity.

The fourth specimen in our possession, representative of the stage under notice, is designated in our list as Specimen Y. The circumstances of our access to this egg are somewhat interesting. Some few years ago one of us received from the Australian Museum a spirit-specimen labelled as the pregnant left uterus of an Ornithorhynchus shot on the Cudgegong River, N.S.W., on August 11, 1884, by Mr. A. G. Hamilton, now of Mount Kembla, N.S.W.

A spirit-specimen of 20 years' standing had not appeared a promising aid to research, especially in view of the delicate character of the egg likely to be found in an only very moderately enlarged uterus. We decided, however, to open up the specimen by a longitudinal cut through the uterus with a sharp razor. There was then disclosed within the uterine canal, and in intimate apposition with its walls, an egg measuring 12 by 10 millims., the egg itself being divided into two somewhat unequal hemispheroidal segments. It appeared to be in a condition of excellent preservation, and contained a quantity of loose and easily evacuated yolk-material. Both segments were then stereophotographed with the aid of the Braus-Drüner camera. A stereograph of the deeper of the two segments, *in situ* in the uterus, is reproduced in fig. 6, Plate 4. In this stereograph the primitive knot may be recognised as a small white spot in the better-lighted area of the concave segment. It was decidedly more prominent here than in Specimen DD, formerly described. We sought in vain for indications of the primitive streak in the vicinity of the knot. On the opposite segment of the egg, however, there was discovered, with some difficulty, a tolerably lengthy portion of a primitive streak. This measured 4·9 millims. in length and ran obliquely to the cut edge of the segment which intersected it very obliquely. This caudal segment of the primitive streak was found to end posteriorly, after widening slightly, by joining the concavity of a crescentic thickening which extended across, at right angles to the course of the streak, for a distance of 1·28 millims. The apparent breadth of the streak itself, a short distance in front of its caudal termination, was about 0·2 millim.

A re-examination of the margin of the other hemisphere of the egg, in the region corresponding to its oblique intersection with the primitive streak, now resulted in the discovery of the anterior segment of the latter, so that we were at length enabled to correlate the two portions of the streak and its surrounding area, which had been separated from one another in the original bisection of the ovum *in situ*. The area of the blastoderm surrounding the primitive streak was recognisable as slightly more opaque than elsewhere, corresponding with the extension of a mesodermal sheet like that which we had previously found in the corresponding situation in the sections of specimens D and DD.

From the above general account of the structural features manifested by the four specimens referred to, it will appear that they all concur in the fact of the differentiation of a primitive streak, and of an area surrounding it on either side, in which the blastoderm is trilaminar; and also in the presence of a "primitive" or "archenteric" knot, quite distinct from the primitive streak and its area. The knot is not only distinct from the streak, but lies at some distance from its anterior end, and the blastoderm in its immediate vicinity is bilaminar merely.

Seeing that the primitive knot contains an archenteric or gastrulation-cavity, more or less typically differentiated, this stage of Ornithorhynchus development may appropriately be termed the "gastrular" stage, the "second phase of gastrulation" having now clearly manifested itself.

(b) *Detailed Account of Anatomical and Histological Characters of Gastrular Stage.*

We may now proceed to offer a more detailed account of the anatomy and histological characters of the gastrular stage.

(a) *General Wall of the Vesicle.*—Exclusive of the primitive knot and of the primitive streak and its surrounding area, the wall of the blastodermic vesicle is bilaminar and quite simple in its constitution. The histological characters of this indifferent bilaminar blastoderm are illustrated in the photomicrograph which is reproduced in fig. 7, Plate 4. The vitelline membrane and shell did not appear in the field, and the section of the delicate ectodermal membrane, here unsupported by its coverings, is wavy in contour and only partly seen in section, partly on the flat, owing to the repeated twisting of the narrow ectodermal shaving. In contrast to the attenuated ectoderm, the thick layer of swollen and yolk-laden entodermal cells is well seen, with their large vesicular nuclei. Fig. 8, Plate 4, is a reproduction of a photomicrograph, by transmitted light, of a bilaminar portion of the wall of the blastodermic vesicle of a stage considerably more advanced than the present one. We have prepared exactly similar photographs from the bilaminar blastoderm of the present gastrular stage, but inasmuch as they are practically identical with that shown in fig. 8, it is unnecessary to reproduce them. The condition represented

in fig. 8 is common to the bilaminar portion of the extra-embryonic vesicle-wall in all of the stages which are specially dealt with in the present paper. It will be observed that over a small area of the field of the preparation, the entodermal layer has been dislodged, and that over this area the relatively closely set nuclei of the ectoderm are alone apparent, the cell-outlines being hardly distinguishable as such. A general somewhat irregular hexagonal outline of the individual cells may, however, be inferred.

Over the remainder of the field it is the huge yolk entoderm cells which are in evidence. These are seen to be loaded with yolk-spheres, whose intracellular relations are further illustrated in fig. 7.

These histological characters obtain throughout the entire extent of the vesicle-wall in the present stage with the exceptions of (*a*) the minute area actually occupied by the primitive knot, and (*b*) the more extensive area traversed axially by the primitive streak. As already indicated, these two areas are not continuous, since in all four specimens of the stage the primitive knot is completely environed by unmodified bilaminar blastoderm having the structure above described. It is situated at a distance of approximately 2–3 millims. in front of the anterior end of the "primitive-streak-area."

It will be convenient to describe (1) the primitive knot, and (2) the primitive streak and its area, as they appear in the "gastrular" stage.

(b) *Primitive Knot.*—This forms an isolated patch of differentiated tissue, abruptly marked off from the wall of the vesicle surrounding it. Its immediate blastodermic environment is bilaminar, and differs in no essential feature from that which forms the general undifferentiated wall of the blastodermic vesicle elsewhere.

In three out of the four specimens we have been able to determine with more or less accuracy the locality of the knot with reference to the anterior limit of the primitive-streak-area. The most accurate determination was made from the sections in the case of our Specimen Q, and its position in this case is indicated in the schematic text-fig. 7, p. 55.

In our previous short paper ('03), there was contained an account of the structural characters of the primitive knot, based especially upon our Specimen DD. That specimen still remains the most generally satisfactory example of what we regard as the typical knot-structure. From the two remaining specimens in which the knot was uninjured, we are able to supply a few further details of organisation; and also to indicate certain individual divergences of structure. These do not, however, in our opinion, affect that conception of the typical constitution, and of the morphological significance of the knot, which was set forth in our former communication.

In its general form the knot is somewhat irregularly oval or oblong, with its shorter axis directed antero-posteriorly. Its dimensions are approximately the same in the three specimens in which it had escaped injury, viz., 0·4–0·5 millim. in

the longer diameter (transverse), and 0·32–0·42 millim. in the shorter (antero-posterior) diameter.

The general structure of the knot in Specimen DD was formerly described in the following terms (*ibid.*, p. 320):—"In mesial section the knot is seen to form a thick and prominent lenticular mass projecting into the cavity of the blastodermic vesicle. It is largely composed of a loose reticular tissue, in which nuclei are only sparsely distributed, and cell-outlines are for the most part invisible. This tissue is thickly dotted with minute yolk-spherules and small vacuoles, and is not limited towards the cavity of the vesicle by any very sharp or clear-cut boundary. This reticulum of the knot is continuous peripherally with the yolk-entoderm of the bilaminar blastoderm around the knot.

"Penetrating the interior of the knot is the archenteric or gastrula-cavity, opening on the surface at the blastoporic aperture near the hinder part of the knot, and appearing in sagittal section as a curved canal passing from the blastopore at first deeply, and then forwards, to end blindly in the more anterior part of the knot. The cavity is lined throughout by a very definite cellular wall.

"Both in front of and behind the knot, the blastoderm is simply bilaminar, with thin ectoderm closely applied to the deep surface of the vitelline membrane. The entodermal cells are large and contain yolk-spherules of varying size and staining reaction and loose yolk-spheres are also found adherent to its deep surface."

To the above description it may now be added that there is recognisable a somewhat indefinite differentiation of the knot-tissue into (*a*) a more peripherally placed cortical or marginal zone, in which the reticular character is more marked, and the nuclei are very few in number; and (*b*) a more central mass which is less reticular and more cellular, and is also freer from yolk-granules. This latter cellular zone includes the cellular wall of the flattened archenteric cavity. The semi-diagrammatic fig. 2 of our preliminary paper shows this cellular wall somewhat too sharply defined, as contrasted with the tissue immediately surrounding it, the more so that little detail is indicated in the latter region. It is nevertheless true that in some sections the line of demarcation between the cellular archenteric wall and the tissue adjoining it is quite clearly defined. As a rule, however, the surrounding tissue is of a more substantial character than the figure would suggest.

We now reproduce in figs. 9 and 10, Plate 4, photomicrographs of two of the more typical sections from the series of sagittal sections through the gastrula- or primitive-knot in Specimen DD. These illustrate in a thoroughly objective fashion the structure of the knot as above described.

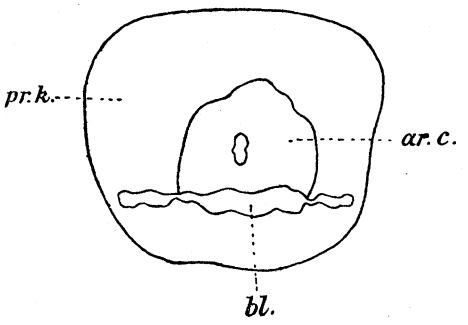
In regard to one or two other points we find it necessary to modify our earlier account of the knot, upon which further light has been thrown by the study of the later specimens.

We formerly stated (**03'**, p. 317) that the yolk-entoderm has been differentiated everywhere as a lining to the cavity of the blastodermic vesicle "except under the

small area of the primitive knot, with whose tissue it is continuous." Examination of sections of the knot in our Specimen Y has shown us (*cf.* fig. 11, Plate 5, and text-fig. 4, p. 51) that the yolk-entoderm is not naturally absent under the knot, but is continued under it as a definite entodermal layer. The appearance of the layer is indeed slightly modified in this situation, but there is no difficulty in recognising it as such, except over a small area beneath the centre of the knot, and it is most probable that its absence here is due to accidental denudation in the case of the knot in Specimen Y. In any case we are compelled to assume that such a denudation has occurred over practically the entire extent of the knot in Specimen DD (*cf.* figs. 9 and 10 with fig. 11). The loose marginal reticular zone of knot-tissue would readily permit of such disintegration and disappearance. Renewed investigation of the relation of the yolk-entoderm to the knot in our original Specimen DD has, in fact, shown us that, in one or two of the sections at least, there are indications of an extension of the yolk-entoderm for a short distance under the peripheral part of the knot, at its anterior margin. Further, we have already seen that in the earlier stage, represented by our specimen "alpha," the layer of definite yolk-entoderm was absolutely complete, and extended right across under that lenticular mass of cells which we must assume to represent the earliest stage in the differentiation of the cellular tissue of the primitive knot.

Unfortunately, the evidence obtainable on this matter, from a study of the sections of the knot in Specimen Q, is of little or no value, inasmuch as the free ventral surface of the knot in that specimen presents an obvious imperfection.

In fig. 1 of our previous paper, we presented a schematic surface-projection of the outline of the knot, showing the blastoporic aperture and the contour of the archenteric cavity. Since then we have plotted out, with somewhat greater accuracy of detail, a plane-reconstruction of the knot. We now reproduce a schematic text-figure surface-scheme (text-fig. 3), revised in accordance with the latter.



TEXT-FIG. 3.—Schematic Plane-reconstruction of the "Primitive" or "Archenteric" Knot (*pr. k.*), with the Blastoporic Aperture (*bl.*) and the Archenteric Cavity (*ar. c.*), in Specimen DD. Magnif. = × 100.

From this it will appear that the archenteric cavity, at its transverse opening on the surface, is really somewhat less wide than it was formerly represented to be. But, though the actual blastoporic aperture is thus reduced in width, a groove-like depression is seen to be continued outwards from it on either side, beyond the actual archenteric limits. Further, a small area is marked out on the surface-projection, corresponding to a limited region, in which the archenteric lumen is obliterated. Since the figure is schematic only, certain irregularities in the peripheral contour of the knot, as a whole, have been omitted.

Fig. 11, Plate 5, represents a photomicrograph of one of the sections through the primitive knot in Specimen Y. Here a portion of the shell is seen in section, closely lined by vitelline membrane, and also, throughout the greater part of its extent, by the thin cellular membrane formed by the ectoderm. It is obvious that the knot has been loosened, and to some extent detached, from its original position with reference to these structures. But the ectoderm is still seen to be connected with the middle part of the knot by reflexion from the deep surface of the vitelline membrane.

It is apparent, in the photomicrograph, that the yolk-entoderm of the vesicle, beyond the region of the knot, has suffered detachment from its natural intimate contact with the ectodermal layer. On the left of the field this detached yolk-entoderm is, on the one hand, continuous with the yolk-entodermal investment of the deep surface of the primitive knot, and on the other hand, is reflected ventrally into a wholly unnatural position under the knot. In this artificially inverted position, it is also seen to be associated with the similarly detached and reflexed ectoderm of the bilaminar vesicle-wall.

This layer may be recognised, in the extreme left of the photomicrograph, leaving abruptly the deep surface of the vitelline membrane, and reappearing in the lower part of the field, where, as already seen, it loosely accompanies the detached and dislocated yolk-entoderm.

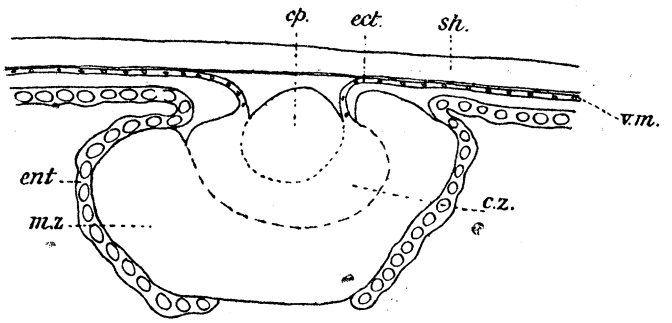
On the right-hand side of the photomicrograph, the structural relations are essentially identical, although less easily recognisable than on the left.

Text-fig. 4 offers a schematic representation of the relation of the blastodermic layers to the primitive knot, as well as of the constitution of the knot itself. The plane of the section-scheme, like that of the section photographed, is approximately transverse as regards the embryonic axis.

Histologically, the characters of the knot are, generally speaking, very similar to those in Specimen DD. Nevertheless, the morphological arrangement of the tissue-elements appears to differ in the two specimens, if not essentially, at least somewhat markedly.

Even more clearly than in the case of the primitive knot of Specimen DD, we can recognise, in that of Specimen Y, a differentiation into zones. The marginal or cortical zone of the knot-tissue (*m. z.* in fig. 11, Plate 5, and text-fig. 4) is

clothed ventrally by the continuation of the yolk-entoderm. This zone exhibits the same coarsely reticular, indefinite, and feebly-staining characters already noted in



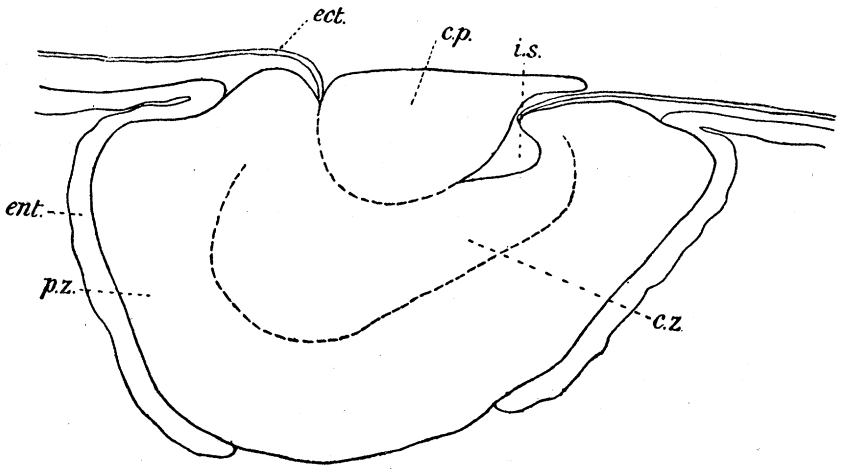
TEXT-FIG. 4.—Scheme of Transverse Section across the Primitive Knot in Specimen Y.
sh., shell; *v. m.*, vitelline membrane; *ect.*, ectoderm; *ent.*, yolk-entoderm; *m. z.*, marginal or cortical zone; *c. z.*, central, more cellular, zone, with *c.p.*, cell-plug. Magnif. = × 128.

Specimen DD. Like it, too, it is poor in nuclei, which are chiefly met with near its entodermal aspect. Yolk-spherules are more scantily present in this zone, and, indeed, in the entire knot, than was the case in the first described specimen. In the sections, this marginal or cortical region of the knot-tissue appears as a crescentic zone, enclosing in its dorsal concavity the more cellular remainder of the knot. This is doubtless homologous with what we have described as the central cellular mass of the knot in Specimen DD. It is separated from the cortical zone by a line of demarcation, which is, in general, recognisable, but is by no means equally clear throughout. Further, this central cellular mass of the knot is again divisible into two areas, viz.: (*a*) a more peripheral, composed of paler cellular material, *c. z.*, with moderately numerous large and clear nuclei; and (*b*) a central area composed of a dense-looking mass, or "plug," of cells, *c.p.*, with smaller and more deeply-staining nuclei. It is this cell-plug which forms the central superficial part of the knot (*c.p.* in the figures). The continuity of the ectoderm with the middle part of the knot, already referred to, takes place around the periphery of this superficial cell-plug, whose surface is thus seen to lie in direct apposition with the deep surface of the vitelline membrane (*cf.* text-fig. 4). (The continuity of the superficial ectoderm of the vesicle-wall with the middle part of the knot, around the cell-plug, is tolerably clearly shown on the left side in fig. 11, Plate 5.) This cell-plug appears very prominently in the photomicrograph in fig. 11.

We find it far from easy so to interpret the tissue-arrangements met with in the present specimen, as to bring it completely into line with the earlier described Specimen DD, which exhibited an evident archenteric cavity. Fortunately, the

structure of the latter is too clear to allow of any misapprehension of its morphological character. Systematic examination of the serial transverse sections of the knot in Specimen Y induces us to believe that this egg had not yet attained the same stage of gastrular development as was manifested in Specimen DD. An archenteric cavity, like that of the latter specimen, does not yet exist in Y.

It is, however, not wholly impossible to interpret the structural characters of the knot in Y in terms of the formation of an archenteric cavity. In text-fig. 5,



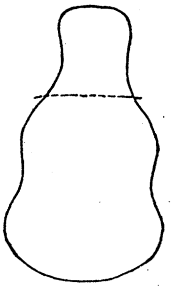
TEXT-FIG. 5.—Schematic Mesial Plane-reconstruction of the Primitive Knot in Specimen Y.

Reference letters as in text-fig. 4. *i. s.*, intercellular space, ? beginning of archenteric cavity. Magnif. = × 250.

there is reproduced a sagittal plane-reconstruction from the transverse sections through the primitive knot of Y. Here the dorsal "cell-plug" is seen represented in its longitudinal extension. Posteriorly, it is more or less continuous with the peripheral portion of the central cellular tissue of the knot above described, with which also the vesicular ectoderm is continuous, whilst, ventrally, it is more definitely limited off from the rest of the knot-tissue. Anteriorly, this cell-plug is seen to be extended forwards as a thin and free tongue-like process, overlapping the junction of the vesicular ectoderm with the cellular tissue of the knot. This latter region must be the homologue of the anterior lip of the blastopore, and it is, in fact, very slightly undermined by a space (*i. s.*), that may possibly represent the commencement of true archenteric invagination.

If the above interpretation be correct, then the cell-plug in question must belong morphologically to the posterior lip of the blastopore, and the posterior segment of the floor of the future archenteric canal. It would thus be in part represented, in

the knot of Specimen DD, by the small and not very prominent mass of cells forming the posterior lip of the blastopore in figs. 9 and 10, Plate 4. The blastopore itself would then be represented, in the knot of Specimen Y, by the line of continuity between the vesicular ectoderm and the knot-tissue around the central cell-plug. And the latter is, in fact, surrounded and limited by a sulcus, the peripheral boundary of which would represent the blastoporic rim. In text-fig. 6, we present a schematic surface-projection of the central plug in relation to the rest of the knot, showing its overlapping anteriorly.



TEXT-FIG. 6.—Schematic Surface-projection of the "Cell-plug" ("*c.p.*" in text-figs. 4 and 5) in Primitive Knot of Specimen Y. Magnif. = × 250.

On the whole, we consider it as at least highly probable that this cell-plug is to be regarded as simply a protruding mass of the cellular tissue of the primitive knot, around which the lips of the blastopore become defined, and which is eventually overlapped, in front and laterally, by the closing in of those lips, whilst, posteriorly, it retains its connection with the posterior lip. This forms a bulging mass, which by and by loses its prominence, and largely disappears.

If our interpretation of the structure of the primitive knot in Y be, on the whole, a correct one, then this blastoderm exhibits an early condition of the second phase of the process of gastrulation, characterised by the inflection of the ectoderm close to its connection with a cellular mass, or "primitive plate." This plate is itself, for the most part, deeply depressed beneath the surface, and, in a sense, actually invaginated, although the hollow of the invaginated cell-plate is, as it were, completely choked up by a protruding cell-plug.

In view of the estimate we have given of the relative degree of organisation of the primitive knot in Specimens DD and Y, we are bound to point out, on the other hand, that, both in absolute size of the egg, and, what is of more importance, in extent of development of the mesoderm in the primitive-streak area, Specimen Y seems to have been somewhat further advanced than Specimens D and DD. We have at present no means of explaining this apparent discrepancy. In any case, we cannot see how the primitive knot in Y could possibly be derived from such a definite gastrula-knot as is found in DD. Moreover, the condition which we find

to exist in the next stage at our disposal, is referable to just such a prior condition as is found in DD, and is not immediately derivable from the condition we have just described in the primitive knot of Specimen Y.

(c) *Primitive Streak and its Surrounding Area.*—We may now turn our attention to the other region of special differentiation which we find to be present in Ornithorhynchus eggs belonging to the "gastrular" stage now under notice. This region is the area traversed axially by the primitive streak. We therefore specifically designate it as the "primitive-streak area," since the complete exclusion from it of the primitive knot precludes its identification with more than the posterior segment of the "embryonic area" or "embryonic shield" of other mammals.

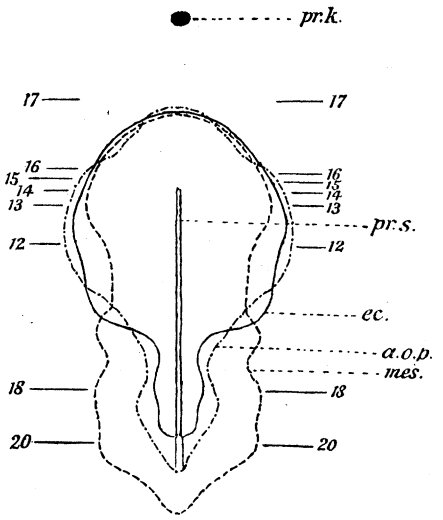
In our preliminary paper it was stated that owing to various circumstances connected with the treatment of the specimens then dealt with it had been impossible to determine with precision the exact orientation of the primitive-streak region. In the two specimens of the same stage subsequently examined we have been able to confirm, and largely to supplement, our earlier observations. In both of these cases, as we have stated, the primitive-streak area was eventually determined. Its form and constitution are well illustrated in the case of Specimen Q, which may therefore be taken as the type-specimen as regards the features of the primitive-streak area. With the condition here displayed, each of the other specimens of the stage is in essential agreement.

When this blastoderm was viewed *in toto* by transmitted light, the linear opacity of the primitive streak was recognised, and the blastoderm in its vicinity showed a very slightly increased opacity. This, however, was only obscurely visible, and no very definite limits could be assigned to it. The differentiation of this region was also distinguishable by reflected light.

The entire region implicated was double-embedded and cut in serial sections transversely to the axis of the primitive streak. From the sections we have prepared a plane-reconstruction-scheme of the primitive streak and its area, together with the primitive knot in its relation to the latter. This schematic figure is reproduced in text-fig. 7, p. 55. In this figure contour-lines have been plotted out, marking the limits of (*a*) an area of entoderm whose cells are much less heavily yolk-laden than those of the entoderm round about it; (*b*) the area of extension of mesoderm; and (*c*) an area of more or less thickened ectoderm, merging, beyond the limit thus indicated, in the attenuated layer of flattened cells, characteristic of the "extra-embryonic" vesicle-wall.

The entodermal area (*a*) whose limit is defined in the figure by the contour-line *a. o. p.* may thus be identified as defining an "area pellucida," over against a surrounding "area opaca." In the sections these two regions are marked off from one another with tolerable abruptness, in the same manner as in the succeeding stage, the "postgastrular," to be described later. That the differentiation in

question was not more clearly manifest during examination *in toto*, is probably due to the fact that the more or less accidental presence of a layer of yolk-spheres, adherent to its deep surface, had masked the distinction between the entoderm of the area pellucida and the surrounding more heavily yolk-laden entoderm.



TEXT-FIG. 7.—Scheme of "Primitive-streak area," together with the "Primitive Knot" in Specimen Q, plotted out to scale from the transverse sections. The numbered lines indicate the planes of sections illustrated in the photomicrographs reproduced in figs. 12–20.

pr. k., primitive or archenteric knot; *pr. s.*, primitive streak; *mes.*, outer limit of mesodermal sheet of "primitive-streak area"; *a. o. p.*, line of junction of area pellucida and area opaca; *ec.*, outer limit of area of thick ectoderm. Magnif. = × 6·25.

The ectodermal contour-line *ec.* is practically merely an isometric contour. Beyond it the ectoderm can no longer be described as substantially thickened. Within it the thickness gradually increases from about 4 μ peripherally to about 11–14 μ axially and in front, and about 8–10 μ axially and behind.

It will appear from the scheme that there is a general coincidence of the regions of the different germ-layers therein mapped out. Further, a striking apparent similarity between this "primitive-streak area" and the early "embryonic area" of a mammalian or avian ovum cannot fail to be recognised. But the notion of any complete morphological correspondence between these is excluded on a full consideration of the facts, more especially that of the present position of the primitive knot, which, in the meantime, lies wholly in front of, and totally unconnected with, the "primitive-streak area."

The more detailed structure of the primitive streak and its area may now be further elucidated with the aid of figures illustrative of its sectional anatomy.

The different planes of section illustrated are indicated by reference-lines in text-fig. 7.

We may begin with a section across the primitive streak at about a millimetre behind its anterior extremity. Fig. 12, Plate 4, is a representation of a photo-micrograph of such a section. Here the characteristic features of a primitive-streak formation present themselves, including a shallow, but definite enough, "primitive groove," superficially. Ventrally, the yolk-entoderm forms a continuous layer of somewhat flattened, and here and there yolk-laden, cells, whose form is most easily distinguishable on the right side of the figure. Beneath this again are a number of free yolk-spheres.

The mesodermal layer on each side can be traced outwards from the cellular tissue of the floor of the primitive groove. The latter tissue forms a slightly depressed median area or narrow cell-plate, which appears as if it were definitely limited, laterally, from the adjoining thick ectoderm. In the section the base of this median cell-plate appears to widen laterally and to become perfectly continuous, beneath the ectoderm, with the mesodermal sheet on each side. In our opinion this appearance is simply a structural expression of the generative activity of the primitive streak as regards the mesoderm, and not of an actual intercalation, into the ectodermal axial line, of an axial strip of cells of alien character, as such a figure as that here reproduced tends to suggest. This appearance of a lateral limitation of a median tract of cells from the definitive ectoderm on either side of it, is one which is familiar to the student of primitive streak structure. Almost identical pictures have been reproduced by various authors. Figures representing what is essentially the same arrangement, and which are quite free from all hypothetical suggestion, are to be found in v. KÖLLIKER'S monograph on the development of the rabbit ('82, figs. 32 (2), 33, and 34). Again, in KEIBEL'S ('93) monograph on the development of the pig, sections through the primitive streak are figured which almost certainly bear the same significance as our fig. 12 (*cf.* his figs. 5, Taf. i, and 24, Taf. ii). These figures have been reproduced by WILL ('95), and by him pressed into the service of the theoretical interpretation of the mammalian primitive streak which he has formulated. The depressed surface of the median cell-plate in our fig. 12, Plate 4, would seem to answer precisely to WILL'S "Mittelfeld" of his "Primitivplatte." An essentially similar view has been adopted by O. HERTWIG ('03, pp. 920 *et seq.*).

The demonstration, however, that there is present in Ornithorhynchus a typical gastrula-knot, with archenteric cavity and blastoporic aperture, contemporaneously with, and wholly independent of, the primitive streak and its area, seems quite incompatible with, *e.g.*, WILL'S conception of the nature of primitive streak structure in mammals.

The apparent individuality of the axial region of the primitive streak as a "Mittelfeld," its limitation from the neighbouring thick ectoderm and its perfect

continuity with the mesoderm, are therefore, in our opinion, to be regarded, at least in the meantime, as conditioned merely by the mechanics of cell-proliferation along the axial region of a mesoderm-producing area.

Fig. 13, Plate 5, represents a section nearly a millimetre in front of the last, cutting across the streak not far from its anterior extremity (*cf.* text-fig. 7, " 13 "). Here the primitive groove is still quite definite, but both the primitive streak itself, and the ectodermal and mesodermal layers in its vicinity, are considerably thickened. The entoderm remains unchanged.

In the next section, fig. 14, Plate 5, which is only a short distance (about 0·2 millim.) in front of the last, important changes have been introduced into the plan of structure. The entoderm is practically unchanged, being still recognisable as a definite layer of cells, some of which are yolk-carrying. But the ectodermal surface shows no trace of a primitive groove, and it now retains its individuality as a thick and independent layer almost right across the median plane. For a short space mesially, however, its connection with the underlying mesodermal sheet is still maintained. In this plane the mesoderm appears as a substantially thickened and tolerably independent layer, whose connection with, and origin from, the ectoderm are hardly at all apparent. Nevertheless the axial portion of the mesodermal sheet is distinctly thicker than the lateral expansions, and this axial portion is not yet wholly emancipated from its connection with the neighbouring ectoderm. This emancipation sets in immediately in front of the plane figured in fig. 14.

Fig. 15, Plate 5, represents a section a very short distance in front of the last. It will be observed that ectoderm and mesoderm are now wholly independent of one another, and that, although the ectoderm is still somewhat thicker over the axial area than further out, there is absolutely no trace in it of any primitive streak differentiation. The mesodermal sheet is also perfectly free and independent of either ectoderm or entoderm. It forms a tolerably compact sheet, thickened for a considerable distance axially, and thinning out somewhat abruptly when traced laterally.

In front of the transverse plane now reached there is a tolerably rapid diminution in the thickness of the constituents of the axial region of the primitive-streak area; and also a rapid loss of the distinction between the axial and the peripheral portions of it.

Thus in fig. 16, Plate 5 (about 0·3 millim. in front of fig. 15), the axial region is only marked out by a slight mesodermal plate-like thickening. The ectoderm is now merely cubical and is practically uniform in character throughout the field.

Almost immediately in front of this plane the axial thickening of the mesoderm wholly disappears, and, as we pass still further forward, the mesodermal sheet itself becomes more and more incomplete until it finally disappears altogether. The

ectoderm also gradually falls off in thickness until it assumes the flattened form characteristic of this layer throughout the unspecialised portion of the vesicle-wall.

Fig. 17, Plate 5, shows a section just in front of the primitive-streak area, in so far as that is defined by its thickened ectoderm. Since, however, the marginal transition from thick to thin ectoderm is not an abrupt one, the layer still exhibits slight traces of that thickened character which it possesses further back. The mesoderm has now entirely disappeared from the section, and the entoderm has regained the thick, yolk-laden character which is distinctive of the "area opaca."

The posterior portion of the primitive-streak area is distinguished by the narrowness of the area of thickened ectoderm surrounding the primitive streak, as indicated in text-fig. 7. This figure also shows that the entodermal contour, marking out the area pellucida, conforms in a general way to that of the ectodermal thickening. The streak itself preserves its typical character, and even increases in thickness as it is traced backwards towards its hinder extremity. The primitive groove, too, is generally met with in the sections, but is not completely continuous. A photo-micrograph of the axial region of a section passing across the narrow posterior portion of the primitive-streak area is reproduced in fig. 18, Plate 6, the precise plane of section being indicated by a reference-line in text-fig. 7. The limit of extension of the mesoderm does not appear in the field; it is found to extend laterally to about the same distance as it does further forwards (*cf.* text-fig. 7), and, therefore, far beyond the limit of the now narrow ectodermal thickening, and the "pellucid" area of the entoderm. Fig. 19, Plate 6, illustrates a portion of the more lateral region of the primitive-streak area from the same section, whose axial region is illustrated in fig. 18. This shows the practically unthickened ectoderm, and the heavily yolk-laden entoderm, and, intercalated between these, the attenuated and imperfect marginal portion of the mesodermal sheet.

Further back, the area of thickened ectoderm becomes still further circumscribed laterally, and there is a marked decrease in the thickness of the primitive streak. The mesodermal cells are seen to be rather sparsely distributed, but they, nevertheless, still extend laterally to about the same distance as in the more anterior planes.

The next section figured (fig. 20, Plate 6) shows still further diminution in the extent and in the thickness of the cubical ectoderm. Here the last remains of the primitive streak thickening appear almost as a simple layer of cells, more or less continuous with the deep surface of the axial ectoderm. From the figure, it might be supposed that, apart from this axial mesoderm of the primitive streak, there was no third layer. The latter is, however, represented, beyond the limits of the figure, by scattered mesodermal cells, extending outwards as far as the mesodermal contour-line indicated in the schematic text-fig. 7.

Immediately behind the plane of fig. 20, all trace of the primitive streak is lost, and the characters of the axial ectoderm and entoderm have now come to resemble

those seen in fig. 19, taken from the lateral region of the "primitive-streak area" further forwards. Scattered mesodermal cells are found as far back as the mesodermal contour limit indicated in the schematic text-fig. 7.

From this illustrated account of the "primitive-streak area" in Specimen Q, it will appear that the tolerably long and typically-formed primitive streak fades away, posteriorly and postero-laterally, into a thin and imperfect mesodermal sheet, which gradually disappears.

Anteriorly, the primitive-streak tissue gradually emancipates itself from its intimate connection and confusion with the axial ectoderm, and is then continued, without break or interruption of any kind, into a broad and solid axial thickening of the mesodermal sheet, which is itself a thick and compact layer (*cf.* figs. 14 and 15) in this anterior region.

The account here given of the sectional anatomy of Specimen Q, is, upon the whole, applicable to the other specimens belonging to the same gastrular stage. Any differences observed were of subordinate importance. It was notable, for example, that the mesodermal sheet in Specimen Y had attained a greater degree of extension than in any other of the specimens of the stage; and the organisation of the area as a whole seemed to be somewhat in advance of that met with in Specimen Q, as above set forth.

Our sections of the "primitive-streak area" in Specimen DD were approximately transverse to the long axis, and the condition there displayed closely resembles that of Specimen Q. Fig. 21, Plate 3, shows a drawing, with the aid of the camera lucida, of a cross-section of the primitive streak in this specimen. Here the structure of the primitive streak may be recognised as identical with that shown in the photomicrograph in fig. 12, Plate 4. In each case we see that the surface of the primitive streak exhibits a tolerably definite axial area or strip corresponding to the "Mittelfeld" of WILL.

In Q this forms the floor of a primitive groove, whilst in DD it is not actually depressed into a groove in the plane figured.

(c) *Summary of Characteristics of Gastrular Stage.*

We may now attempt to summarise the characteristics of the developing ovum of Ornithorhynchus at the stage which we here denominate the "gastrular" stage, as follows:—

(*a*) The egg has a diameter of between 9 and 12 millims. Its shell measures from 0·022—0·03 millim. in thickness. The "vitelline membrane" (? zona pellucida) is thin and delicate.

(*b*) The ovum itself has undergone conversion into a large blastodermic vesicle through the complete extension around its interior of the two primary germinal layers, in the shape of a delicate blastodermic membrane, and by liquefaction of its contents.

As was stated in our preliminary paper ('03), "one can, without hesitation, homologise the interior of the vesicle with the sub-germinal cavity of a Sauropsidan egg, extended so as to include, by liquefaction, the whole of the yolk itself. Ornithorhynchus may, indeed, be said to afford an actual demonstration of the transformation of a Sauropsidan sub-germinal cavity into the cavity of a typical mammalian blastodermic vesicle, thus supporting KEIBEL'S view as to the correspondence of these cavities" (*loc. cit.*, p. 317). The first beginnings of this process in Ornithorhynchus are illustrated in SEMON'S figs. 36 and 38, from his Specimen O_3 ('94).

(*c*) The completed wall of the blastodermic vesicle thus originating is nowhere less than two-layered. Its innermost layer consists of a complete sheet of yolk-entoderm cells ("secondary," or "cenogenetic," entoderm; "paraderm"; "lecithophore," etc., of various authors), which everywhere lines the cavity. The outermost, or ectodermal, layer is composed throughout, for the greater extent of the vesicle-wall, of extremely flattened and attenuated cells. These are so closely applied to the deep surface of the vitelline membrane as to be well-nigh invisible in sections.

(*d*) There exists an area of the wall of the vesicle, of somewhat elongated or oval form, in which the constitution of the blastodermic membrane exhibits greater complexity and specialisation. Here the ectoderm is no longer an attenuated membrane, but is considerably thickened, and consists of cubical or columnar cells. Throughout this area, also, the blastoderm is trilaminar, owing to the presence of a sheet of mesoderm, complete throughout the greater part of the area, and extending though in less compact and complete condition, beyond the limits of the area of thickened ectoderm.

(*e*) Traversing the long axis of this area there is found a linear primitive streak of quite typical constitution. This is of considerable length, extending for a distance of 5—6 millims., and occupying the greater part of the length of the area referred to in the preceding paragraph. This entire region we therefore designate the "primitive-streak area."

As the anterior portion of the primitive streak is traced forwards, it is found to fade away as a gradually diminishing axial thickening of the mesodermal layer, thinning away into the latter both anteriorly and laterally. Ere it disappears, this axial thickening of the mesoderm presents a certain superficial resemblance to a "head-process of the primitive streak," but it is widely different in character from the structure ordinarily described under that name.

(*f*) Wholly apart from, and independent of, the primitive streak and its area, there is also present in the blastoderm, at the stage under consideration, a genuine "primitive knot," greatly resembling that found in reptiles. This lies at a distance of from 2—3 millims. in front of the "primitive-streak area." It is a small and definite isolated knob, bulging somewhat into the cavity of the vesicle. It is more

or less oval or oblong in form, and measures about half a millimetre in diameter. The primitive knot is separated from the nearest part of the periphery of the primitive-streak area by unspecialised bilaminar blastoderm, by which the primitive knot is, indeed, completely environed.

(6) "Postgastrular Stage," with Differentiation of Elongated "Archenteric Plate." (Specimens E, F, P, and PP.)

The next stage of Monotreme development, represented in our collection, is one which we have provisionally termed "postgastrular," as succeeding the "gastrular stage" described in the preceding sections.

It is true that the term "postgastrular" is rather vague, and does not of necessity imply any final limit to the processes which characterise it.*

We employ it, however, as applicable to the structural condition which we find to supervene closely upon the completion of the second phase of gastrulation. This condition includes the development, from the primitive knot, of the so-called "head-process," together with various other phenomena associated with this, either causally or contemporaneously. This phase of development is deserving of special recognition as constituting a new era, for, with its onset, the process of "notogenesis" is initiated, and the proper axis of the future embryo (Minot's "primitive axis") is laid down.

(a) *Description of Individual Specimens of Postgastrular Stage.*

Four eggs have come into our hands which belong to this postgastrular stage. These appear in our catalogue under the designations "E," "F," "P," and "PP," respectively. Specimens P and PP were twin eggs. The former may be selected as the type-specimen for purposes of general description.

This egg was received unopened and fixed *in toto*. Its diameter was 12·5 millims. Fortunately, it was possible in this case to recognise through the shell, after clearing in cedar oil, a vague indication of some linear differentiation in the blastoderm. It was therefore possible to open the egg in a determinate fashion and without injury to what proved to be the embryonic hemisphere of the ovum. The fluid contents of the vesicle included flocculi of coagulum and was clouded by disseminated yolk-spheres.

Fig. 22 (A and B), Plate 6, represents a stereophotomicrograph (at four diameters) of the concave (ventral, entodermal) aspect of the embryonic hemisphere of the opened vesicle. The blastoderm still lines the hemisphere of the shell and

* A similar charge of vagueness may be brought against our employment of the term "gastrular" as descriptive of the antecedent stage. On our own showing, indeed, the term "gastrular stage" should read, strictly, "completed gastrular stage," inasmuch as both phases of the process are now manifest in the ovum. The simplified term seems to us adequate and not really misleading.

vitelline membrane. (This figure should be viewed with a hand-stereoscope.) In the fundus of the concavity, where the focussing is clearest, there may readily be recognised the long primitive streak, whose anterior end is now in perfect contiguity with the "primitive knot." This latter structure is very obvious by reason of its greater opacity. It forms a small oblong thickening, limited by a tolerably sharp contour, posteriorly, where it is in contact with the anterior end of the streak; but, anteriorly, showing a quite imperfect demarcation from its less opaque anterior, axial, prolongation—the so-called "head-process." Only a small extent of the latter structure is visible in the stereograph owing to the relatively acute curvature of the egg-hemisphere.

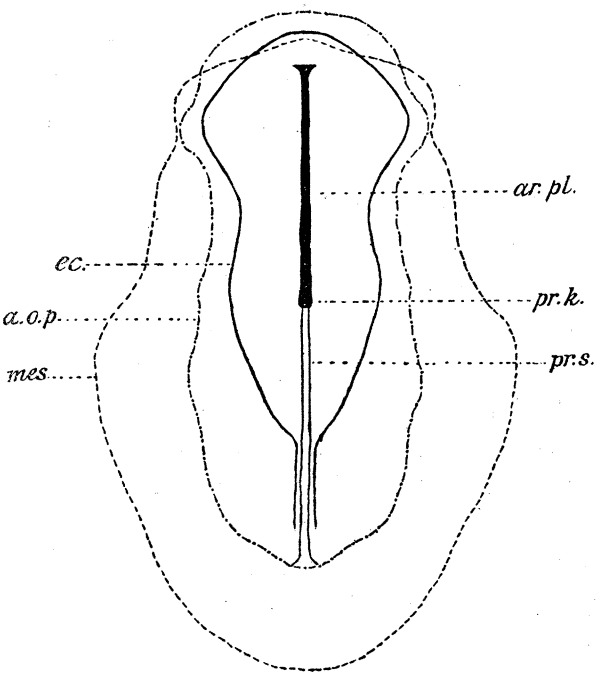
Owing to the practical impossibility of separating the blastoderm from the shell without injuring the former, our operations in the way of examination and photography of the object as a whole were confined to the intravesicular surface of the blastoderm. This technical disability greatly restricted the possibilities of our recognition of the precise surface-differentiation of the embryonic region. A tolerably adequate conception of its organisation was only, in fact, obtained after correlation of the superficially recognisable features with those of the serial transverse sections made from the specimen later on.

The accompanying text-fig. 8 was drawn after a schematic plane-reconstruction from these serial sections, and this has served as a guide in the interpretation of some of the surface-features which appear in our photographs. It may therefore prove advantageous to begin our account of the anatomy of Specimen P with an explanation of this figure.

Extending along the median line of the figure is seen the axial differentiation already referred to, consisting of primitive streak, primitive knot, and so-called "head-process." This latter, as will appear from our subsequent accounts of the sectional anatomy of this stage, is morphologically an "archenteric plate," widening out somewhat at its anterior extremity to form the so-called "protochordal expansion." At its posterior extremity the primitive streak also widens out into a somewhat triangular thickening, which answers to the "Caudalknoten" figured by BONNET ('01, Taf. xxxii, fig. 18, and Taf. xxxiv–v, figs. 20 and 22) in the dog; and also, earlier, by KÖLLIKER ('82) in the rabbit. In the present case the great length of the total axial differentiation is a notable feature of the embryonic region. It measured 11·3 millims., of which 5·8 millims. were occupied by the primitive streak and 5·5 millims. by the "head-process," along with the relatively insignificant primitive or HENSEN'S knot.

The scheme reproduced in text-fig. 8 further shows three contour-lines of similar character to those mapped out in the scheme illustrating the previous stage (text-fig. 7). Of these lines the innermost represents an isometric contour indicative of the limit of the thickened and cubical ectoderm. The area thus enclosed is broad and expanded in front, narrowing in the middle region, and then tapering away

pretty rapidly behind into a pointed extremity. This extremity is, however, continued backwards as a very narrow strip on either side of the primitive streak in its posterior half, which would otherwise lie outside of the area of cubical ectoderm.



TEXT-FIG. 8.—Scheme of Embryonic Region of Specimen P, plotted out to scale from the serial transverse sections.

ar. pl., archenteric plate; *pr. k.*, primitive or archenteric knot; *pr. s.*, primitive streak; *mes.*, outer limit of mesodermal sheet of "primitive-streak area"; *a. o. p.*, line of junction of area pellucida and area opaca; *ec.*, outer limit of area of thick ectoderm. Magnif. = × 6·25.

Peripherally to the isometric ectodermal contour is another contour-line marking the inner limit of the thickened and heavily yolk-laden entoderm. It is true that, even within this boundary, the entodermal cells are by no means free from yolk-spheres in their interior, but they no longer possess the heavily yolk-laden character uniformly displayed beyond the contour-line in question. This line indeed marks a tolerably abrupt transition in character, and may be regarded as marking off an "area pellucida" from an "area opaca."

The differentiation just noted corresponds to that which is familiar in the avian blastoderm, and is entirely different from that of the "area opaca" described by BONNET ('01) in the blastodermic vesicle of the dog. This latter has not only quite another significance, but also a different topographical position.

The outermost of the three contour-lines shown in the scheme marks the limit of extension of the mesoderm. The mesodermal area thus mapped out falls short of

the anterior limit of the area pellucida, and even very slightly of the anterior limit of the area of thick and cubical ectoderm.

The remainder of the wall of the blastodermic vesicle, beyond the region represented in the text-figure, is simply bilaminar, and does not differ in structure from that found in the preceding gastrular stage. The surface-view of a portion of it, as seen by transmitted light under moderately high-power magnification, is also practically identical with that shown in the photomicrograph reproduced in fig. 8, Plate 4, representing a portion of the bilaminar blastoderm from an egg belonging to a somewhat later period of development.

In those mammalian embryos of which, at a corresponding stage, we possess the most adequate descriptions, *e.g.*, rabbit, pig, and dog, the differentiation of a definite area of thick ectoderm to form the so-called "embryonic shield" seems to be a constant phenomenon. In these forms the original oval form of the shield gives place to a more elongated one, soon becoming shoe-sole-shaped. When this occurs the posterior narrower part of the shield is found to possess a somewhat thinner ectoderm than its more anterior portion (*cf.* BONNET, '01). Partly, at least, in virtue of this distinction, the posterior narrower part of the shield comes to assume a somewhat different appearance to the rest of it; and, as the development of the shoe-sole-shaped shield proceeds, this differentiation extends forwards from the posterior region along its lateral margins (*cf.* BONNET, *loc. cit.*, Taf. xxxiv–v, figs. 20–23). BONNET identifies the marginal region thus arising as corresponding to the "parietal zone," whilst the remainder of the shield, which is thicker, more opaque, and more prominent, is stated by him to correspond to the "Stammzone" (paraxial zone) on either side of the axial structures. "Parietalzone" and "Stammzone" are, of course, at bottom, mesodermal differentiations; but they would appear also to correspond to areas of early differentiation of "shield ectoderm" in, *e.g.*, the dog. Now, in Ornithorhynchus, at the stage at present under consideration, the differentiation of cubical ectoderm is confined to an area which strictly coincides with the "Stammzone" in the dog, as will be evident from a comparison of BONNET'S fig. 22 (*loc. cit.*) with our text-fig. 8. And, over the region which would correspond to the "Parietalzone" in BONNET'S figs. 20–23, we have in Ornithorhynchus only flattened squamous ectoderm, which passes over on each side, without any distinction or demarcation, into the general extra-embryonic ectoderm.

If, then, we proceed to define an "embryonic shield" in terms of thickened cubical ectoderm in Ornithorhynchus, we find that the area so defined coincides—not with the entire shield as differentiated in the other mammalian blastoderms referred to—but only with the area of the "Stammzone." The outer limit of our "area pellucida" is seen in text-fig. 8 to lie not very far beyond the limits of this ectodermally defined "Stammzone."

For the reasons just stated we are of opinion that the employment of the term "embryonic shield" in the case of Ornithorhynchus is liable to lead to inaccurate

comparisons. We shall therefore simply employ the term "embryonic area," in the meantime, to signify that region of the blastoderm which we have defined as an "area pellucida."*

In the stereographic fig. 22 the differentiation between "area opaca" and "area pellucida" cannot be distinctly recognised. The triangular posterior expansion of the primitive streak (so-called "Caudalknoten") appears as if it merged in the edge of the area opaca. In reality, however, as the serial sections show, the widened hinder extremity of the primitive streak is continuous with a crescentic mesodermal thickening which happens to coincide in position with the edge of the area opaca constituted by the yolk-entoderm. This crescentic mesodermal thickening is, however, not distinguishable, as such, on mere superficial examination of the blastoderm, at the present stage, *e.g.*, in the stereograph in fig. 22. Here the marginal part of the area opaca exhibits a somewhat indefinite zone of greater opacity, horse-shoe-shaped in form, which skirts the hinder half, or more, of the area pellucida, running parallel with, but not quite reaching, its margin. Posteriorly, this horse-shoe-shaped zone really passes behind the crescentic mesodermal thickening in which the primitive streak ends; but, on surface view, in the present stage, these two crescentic opacities are indistinguishable from one another and appear as one. In later stages they become quite separate. Comparison with the specimens of the more advanced stages in our possession, as well as with serial sections of specimens of the present stage, shows that the somewhat indefinite, horse-shoe-shaped zone now described is the site of the earliest differentiation of vaso-formative tissue, and may therefore be appropriately termed the "protangioblastic zone."

It has already been stated that the area of cubical ectoderm shown in text-fig. 8 corresponds, in our opinion, with the "Stammzone," *i.e.*, the paraxial zone of the future embryo. But it is, of course, the organisation of the mesoderm which actually determines the differentiation of the paraxial zone in the mammalian embryonic area. And there are already present, in the embryonic area of this postgastrular stage, indications of the appearance of such an organisation of the mesoderm. No attempt has, however, been made to delineate the outlines of this incipient organisation in the schematic text-fig. 8. But in viewing the entodermal aspect of the blastoderm, as shown in the stereograph in fig. 22, and still more prominently in the photomicrograph reproduced in fig. 23, Plate 6, taken at a somewhat higher magnification (10 diameters), there may easily be discerned, within the

* In his paper on the development of the sheep, BONNET ('84) shows, in his fig. 51, Taf. xi, a representation of a blastoderm which offers a close parallel to the postgastrular stage in Ornithorhynchus, alike as regards the axial and peripheral differentiation of the embryonic region. BONNET'S text description of this figure is, however, not only in disagreement with the conception of it which we derive from our study of the Monotreme blastoderm, but it does not harmonise with that interpretation of the figure which is forced upon us by the consideration of later observations on the mammalian blastoderm by BONNET himself, as well as by other observers.

area pellucida, an elongated area characterised by its slightly darker shading. This area may be traced outwards for a certain distance on either side of the axial line of the embryonic area, except in the vicinity of the posterior half of the primitive streak. A comparison with text-fig. 8 will suffice to show that the shadowy area in question, so far as visible in figs. 22 and 23, coincides more or less closely with the area of cubical ectoderm mapped out in the text-figure.

The slight increase of the opacity of the elongated region of the area pellucida, here described, is doubtless in part conditioned by the greater thickness of the ectoderm overlying it. But it is not wholly due to this factor, but partly to a slight increase in thickness of the mesodermal sheet in the region concerned. The coincidence between these areas of thickened ectoderm and mesoderm is tolerably close, but it is not quite perfect. Thus, posteriorly, the paraxial mesodermal area, as we may now term it, is somewhat wider than the overlying "shield-ectoderm." The area of the latter tapers away behind the plane of the primitive knot, and practically ends opposite the middle of the primitive streak. The area of paraxial mesoderm, on the other hand, widens slightly in the plane of, and immediately behind, the primitive knot. It cannot, however, be traced backwards, as such, much beyond the middle of the primitive streak, in which neighbourhood the paraxially thickened mesoderm is continued into the general mesodermal sheet, both laterally and postero-laterally. The limits here referred to are only rather vaguely indicated in the photographs, in which also the lack of definiteness is intensified by the presence of minor irregularities of the surface of the blastoderm. But there can be no question as to the genuineness of the distinction in structure which has been here noted.

In the photomicrographs, figs. 22 and 23, which show the deep aspect of the area, there may be seen crossing the paraxial zone obliquely, on either side of the archenteric plate or "head-process," a series of alternating dark and light bands. On the apparent left side four dark bands, with three intervening light ones, may be recognised; whilst, in the more confused out-of-focus region in front, indications of possible additional ones may be seen.

Whether or not these are indications of an early metameric arrangement of the paraxial mesoderm we have been unable to determine with certainty either by surface or sectional examination. That they are not neuromeric is rendered certain by their present extension outwards as far as the present limit of the paraxial mesoderm, and beyond that, which will subsequently prove to be the outer boundary of the medullary plate.

Specimen P is the only specimen of the stage in which we have been able to recognise such indications of mesodermal segmentation. In the other specimens the conditions of observation were less favourable for low-power examination under the dissecting-lens and for surface-photography. In it alone had all crumpling and disturbance of the natural position of the blastoderm been, practically completely,

avoided. It has already been mentioned that in this instance the general lie of the embryonic axis was detected through the shell, in the unopened egg, after clearing in cedar oil. In consequence of this recognition we were able to preserve the embryonic area undisturbed, and in almost perfect apposition with the shell, throughout all the subsequent processes of preparation, up to the final embedding and sectioning. The scheme in text-fig. 8, founded upon reconstruction from this specimen, may therefore be taken as representing with tolerable accuracy the normal proportions of a perfect embryonic region in the postgastrular stage in Ornithorhynchus.

Specimen PP was the twin egg to Specimen P. Its condition in respect of fixation was excellent, but it had undergone a certain amount of distortion from partial collapse whilst in the hands of our collector. The somewhat creased and crumpled condition of the delicate wall of the blastodermic vesicle, revealed upon opening the egg, was distinctly unfavourable to satisfactory surface-examination. It was, however, easy enough to orientate the embryonic area, and we were thus able to obtain from it a practically complete series of transverse sections of the axial region, with the exception of the hindmost part of the primitive-streak region.

Specimen E was likewise an intra-uterine egg, and measured 13 by 11 millims. Though of the same general stage of development as the preceding specimens, it appears to be, in some respects at least, slightly in advance of them.

The egg had been fixed, *in globo*, in picrosulphuric acid by the collector, and was received by us unopened. In this case no trace of embryonic differentiation was visible externally, and the opening up of the egg was therefore necessarily a random procedure. Unfortunately, the blastodermic membrane had not everywhere retained its intimate apposition with the vitelline membrane and shell as in Specimen P; and, as a result of this, the most anterior portion of the embryonic area was destroyed in the manipulation involved in the opening up of the egg. The partial detachment had also produced a certain amount of permanent creasing.

Fig. 25, Plate 7, represents a photomicrograph by transmitted light, at a magnification of 9 diameters, of the ectodermal aspect of the embryonic region of this specimen. The overlying shell and vitelline membrane, which had become detached in the course of the preliminary operations, were removed from the surface.

The axial differentiation in this specimen is practically identical with that present in Specimen P. The primitive streak measured 5 millims. in length. Probably we should not err in adding 0·5 millim. in compensation for the creasing which has occurred in its length. The primitive knot forms a short dark oblong area, 0·35 millim. in length. This is sharply differentiated from the anterior extremity of the primitive streak which is in contact with it. It also appears as if it were abruptly differentiated in front from the third of the axial constituents, viz., the

archenteric plate or "head-process." It is, nevertheless, directly continuous with the latter, and the abrupt demarcation is merely due to a sudden diminution in thickness at this point, involving a loss of opacity in the photograph by transmitted light. The archenteric plate measures only 2·40 millims., owing to deficiency of the anterior part of the embryonic area in the prepared specimen, which was accidentally cut away. Its total length is therefore uncertain.

Owing to the detachment of the shell and vitelline membrane, it was possible in this case to examine the upper or ectodermal aspect of the embryonic region.

By reflected light, the upper surface of the primitive knot showed as a somewhat prominent white spot, with a superficial indentation close to its hinder margin. This posterior margin of the knot was sharply limited off from the streak behind it. On the other hand, the anterior end of the knot appeared to pass over into the "head-process," without any distinct demarcation on surface-view.

In its posterior third, the upper surface of the primitive streak was distinctly grooved. Immediately in front of this the groove was obliterated, but it reappeared in the anterior half, though shallower than posteriorly. It again shallowed out, and disappeared completely, before the hinder boundary of the primitive knot was reached.

The expansion of the posterior extremity of the primitive streak, to form a "caudal knot," was less evident in the case of this specimen, on surface observation only. But both it, and the mesodermal crescent in which it joins, were recognisable in the serial sections.

As in the type-specimen of the stage, an embryonic or pellucid area was definable. The shade-contrast between this and the surrounding area opaca was, however, less marked in this case, when viewed by transmitted light. Nevertheless, the serial sections show the same distinction between the entodermal cells of the two regions, as regards their yolk-laden character, as was noted in the case of Specimen P. As in the latter specimen, the margin of the area opaca, posteriorly, is barely distinguishable from the opacity of the mesodermal crescentic thickening, in which the hinder end of the primitive streak merges.

The zonular thickening of the area opaca, skirting the periphery of the embryonic area posteriorly and laterally, which we have above identified as a "protangioblastic zone," is even slightly more definite in Specimen E.

In this specimen the "paraxial zone" of the embryonic area was somewhat better defined, and more sharply contrasted with the parietal region of the area, than was the case in the type-specimen.

The further characteristics of this specimen will be considered in connection with the sectional anatomy of the stage.

Specimen F was an egg which was received from the collector's hands after fixation, but ruptured, and partially collapsed. Its condition proved to be unfavourable for histological purposes owing to faulty fixation.

The portion of the wall of the vesicle containing the embryonic area, with vitelline membrane and shell adherent, was separated and photographed by transmitted light. We consider it unnecessary to reproduce the photographs.

In them the paraxial region of the embryonic area is even more sharply defined than in the foregoing cases. The cephalic portion of it is visible as a tolerably expanded region (*cf.* text-fig. 8, p. 63). From this the narrower paraxial zone of the trunk region extends backwards, expanding slightly opposite the primitive knot and the anterior portion of the primitive streak, and there tapering into the lanceolate posterior terminal part, as illustrated in text-fig. 8. As in the previous specimens, the posterior tip of this leaf-like ending reaches to about the middle of the primitive streak, so that, here also, about half of the latter is included in the paraxial region, whilst the remainder is prolonged backwards, through the parietal region of the area pellucida, to the edge of the area opaca.

Axially, the archenteric plate appears by transmitted light as a clear band, 0·1 millim. in breadth, extending forwards for 3·5 millims. from a primitive knot which measured 0·28 millim. transversely. Behind the knot the primitive streak is found to extend backwards for a distance of 2·8 millims., of which a length of 1·5 millims. lies within the paraxial part of the embryonic area. From these measurements, it appears that there is a wide discrepancy between this specimen and those detailed above, as regards the longitudinal extent of the axial differentiation. There was probably a very considerable amount of shrinkage in the case of Specimen F. Its ectoderm was throughout swollen and vesicular-looking, and entirely unlike that of our properly conserved specimens.

If an attempt be now made to compare the externally visible features of the organisation of the Monotreme blastoderm in the "gastrular" and "postgastrular" stages respectively, the following conclusions may be arrived at:—

The area of trilaminar differentiation has, in the later of these two stages, undergone considerable increase in size. The "primitive-streak area" of the earlier, gastrular stage has, by a remarkable extension, become converted into a more or less typical "embryonic area." It has undergone a general enlargement and extension, but this general growth is of quite subordinate importance. The most conspicuous and important extension has been the accession to it of a new region anteriorly. This has probably taken place through an extremely rapid extension forwards of those processes of germ-layer-differentiation by which the "primitive-streak area" was itself evolved, in the antecedent stages of development. This new, and relatively rapid, forward extension from the primitive-streak area, would appear to have surrounded, and, as it were, engulfed, the primitive knot. In the gastrular stage the primitive knot was present as a small and completely isolated mass, lying considerably in front of the anterior boundary of the primitive-streak area, and environed by undifferentiated bilaminar blastoderm, like that constituting the

non-embryonic vesicle-wall. It is now, in the later (postgastrular) stage, included in the central region of the greatly elongated "embryonic area," whose posterior segment now represents, after a fashion, the earlier "primitive-streak area."

It must not be forgotten, however, that the primitive knot has played no merely passive *rôle* during the occurrence of these developmental phenomena. On the contrary, it has itself undergone an enormous elongation, giving rise to the lengthy "head-process," whose true morphological character is more adequately expressed in the designation, "archenteric plate."

It is further to be observed that the developmental processes, above summarised, have in some way co-operated in bringing about a *rapprochement* between the primitive knot and the anterior end of the primitive streak, two structures which are originally comparatively remote from, and independent of, one another.

Discussion of the probable method by which this result is attained must be reserved for a later section of the paper.

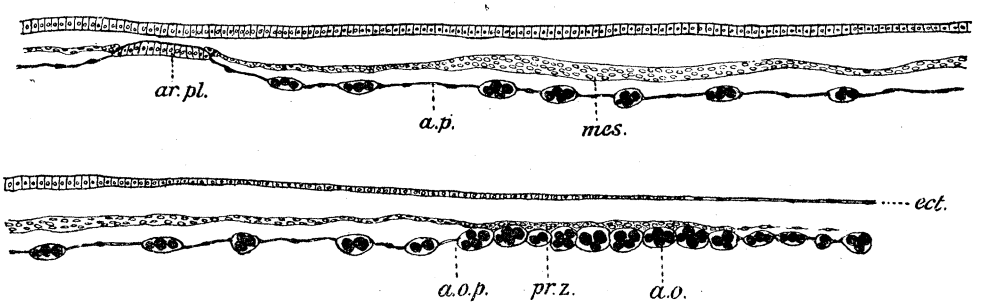
(b) *Sectional Anatomy and Histology of the Postgastrular Stage.*

In our description of the histological characters and sectional anatomy of the postgastrular stage, Specimen P will again serve as the type-specimen, so far as the axial region is concerned.

(a) *General Wall of Vesicle.*—It has already been stated (p. 64) that the structure of the wall of the blastodermic vesicle, away from the area of special embryonic differentiation, is closely similar to that of the preceding gastrular stage. Fig. 24, Plate 6, is a photomicrograph of a section of the bilaminar blastoderm beyond the limits of extension of the mesodermal sheet. A comparison of this figure with fig. 7, Plate 4, from the previous stage will illustrate the practical identity of the two stages as regards this extensive region of the wall of the vesicle. Reference may again be made to fig. 8, Plate 4, illustrating a surface-view of the entirely similar bilaminar blastoderm from a still later stage of development, photographed by transmitted light. The figures show that the ectoderm of the bilaminar area consists of flattened cells forming a much attenuated membrane. Where this happens to be retained in the sections in intimate apposition with the vitelline membrane, its cells are frequently somewhat difficult to distinguish on account of their tenuity. As the outer limit of the "protangioblastic zone" is reached, the ectoderm becomes slightly more obvious in the sections owing to a faint increase in the thickness of its cells (text-fig. 9, p. 71). But it still remains relatively a thin membrane until the margin of the region already defined as the "paraxial zone" is reached. Here there occurs a tolerably abrupt increase in the thickness of the cells of the ectoderm, which now become definitely cubical.

(b) *Sectional Anatomy of Embryonic Region.*—The general sectional anatomy of the trilaminar region of the blastoderm, including, as it does, the region of embryonic differentiation, is diagrammatically illustrated in text-fig. 9. This figure

was drawn from one of the transverse sections of Specimen E, from the region in front of the primitive knot, and at right angles to the archenteric plate. The outer limit of the figure practically coincides with the lateral marginal limit of the mesodermal sheet.



TEXT-FIG. 9.—Semi-diagrammatic Transverse Section across Embryonic Area of Specimen E, passing through the Archenteric Plate.

a. p., entoderm of area pellucida; *a. o.*, entoderm of area opaca; *ar. pl.*, archenteric plate; *mes.*, mesodermal sheet; *pr. z.*, "protangioblastic zone"; *a. o. p.*, junction of area pellucida and area opaca; *ect.*, ectoderm. Magnif = about × 75.

From a comparison of text-figs. 8 and 9 the general characters and relations of the germ-layers in, and immediately around, the embryonic or pellucid area may be realised. The characters of the ectoderm visible in text-fig. 9 have just been remarked upon. The entoderm is seen to be differentiated into "area pellucida" and "area opaca" by the much closer packing together of the yolk-laden cells in the latter region, whose inner limit is marked by the lettering "*a. o. p.*" In the medial direction the entoderm of the area pellucida comes to an end by effecting a junction with the lateral edge of the archenteric plate.

The mesodermal sheet shows an extensive, thickened, "paraxial zone" subjacent to the thickened and cubical ectoderm. Immediately beyond the lateral limit of the latter it begins to thin out into the "parietal" sheet, which extends beyond the limit of the area pellucida, and finally fades away altogether, peripherally to the outer limit of the "protangioblastic zone." It is obvious from the figure itself that what we have termed the "paraxial" mesoderm is a tolerably extensive zone, somewhat irregular in its thickness. Thus, it thins out towards the lateral margin of the "archenteric plate," with which it is contiguous. It also thins somewhat when followed in the lateral direction, to thicken again before the outer margin of the more cubical ectoderm is reached. From this point it pretty rapidly thins away into the "parietal" mesoderm. The latter portion of the sheet, just peripherally to the inner limit of the area opaca, undergoes a slight amount of thickening, and, for a certain stretch, it becomes intimately attached to the underlying entoderm in a rather special manner. This area of specially intimate ento-mesodermal attachment represents the "protangioblastic zone" formerly described as present in the blastoderm at this stage.

It may be added that our determination of the outer limit of the "paraxial" region has been verified and checked by comparison with a later stage of the embryonic region, in which that zone is more evidently established.

The anatomy of the axial structures of the embryonic area may now receive somewhat more detailed consideration.

(c) *Primitive Streak.*—The histological characters of the primitive streak of Specimen P are illustrated in the drawing in fig. 26, Plate 3. The appearance seen in this figure is repeated with little variation throughout the whole of the lengthy primitive streak until within a short distance of its hinder extremity. It will be observed that the cubical ectoderm of the "Stammzone" merges medially into an axial strip of more irregular cells. Along this strip the distinction between ectodermal and mesodermal cells is in abeyance. The figure thus illustrates the same condition as was shown in fig. 21, Plate 3, from the previous stage of development. In both cases the entoderm runs beneath the entire streak-region as a continuous sheet.

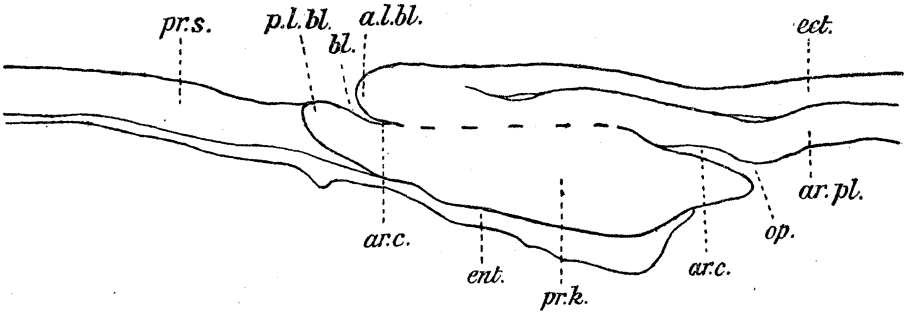
Fig. 27, Plate 3, is a drawing of a section across the posterior extremity of the primitive streak, at the edge of the area opaca, and where it has widened out to form the so-called "caudal knot." Here the streak-tissue is somewhat more massive, but otherwise there is no essential change in its organisation.

Fig. 28, Plate 3, represents a drawing of a section made at right angles to the median line, and behind the primitive streak and the crescentic mesodermal thickening connected with it. In this plane the ectoderm, though still showing a slight amount of thickening, is wholly free from the underlying mesodermal sheet, which here covers the thick entoderm of the area opaca.

(d) *Primitive Knot.*—The anterior extremity of the primitive streak has already been seen to lie in intimate relation with the primitive knot. Even in surface-view it was apparent that the posterior boundary of the primitive knot is sharply distinguishable from the streak (*cf.* fig. 23, Plate 6). And study of sectional anatomy shows that the tissue of the knot is, in fact, distinguishable from that of the streak immediately behind it.

Text-fig. 10, p. 73, will afford a general conception of the axial relationships of the parts in question. This figure is a schematic reconstruction of the median plane of the knot, etc., of Specimen P. The scheme was plotted-out to scale, from the serial transverse sections. The striking resemblance offered by this scheme to representations of the same region in various reptilian embryos cannot fail to be remarked.

The middle, and major, part of the figure represents a median section of the primitive knot, which forms a slight prominence upon the dorsal or ectodermal aspect. Behind this bulging there appears on the surface an aperture, blastoporic in character, and bounded by anterior and posterior "lips," the former of these being prominent and rounded. This blastoporic aperture leads into a flattened



TEXT-FIG. 10.—Schematic Mesial Plane-reconstruction of Archenteric (HENSEN'S) Knot, and adjacent Regions in Specimen P.

pr. k., archenteric or HENSEN'S knot; *pr. s.*, primitive streak; *ect.*, ectoderm; *ent.*, yolk-entoderm; *ar. pl.*, archenteric plate; *op.*, opening of archenteric cavity into cavity of blastodermic vesicle; *ar. c.*, archenteric cavity; *a. l. bl.*, anterior lip of blastopore; *p. l. bl.*, posterior lip of blastopore. Magnif. = × 200.

cleft-like canal which appears shortly to end. Sections made parallel with the median plane would, however, show various irregular continuations forwards, until the canal gradually reappears medially, and finally opens into the cavity of the blastodermic vesicle. The tissue of the massive knot is prolonged forwards, from the region around the blastoporic aperture, to constitute the parietes of the imperfect and irregular canal, as far as the ventral opening of the latter. Beyond this ventral opening the floor of the blastoporic or archenteric canal is lacking. Its roof or dorsal wall, however, is prolonged forwards for a relatively very great distance in the form of the axial "archenteric plate." It is this which is visible as the "head-process," on surface-examination.

As far forwards as the ventral opening of the blastoporic canal the primitive knot is clothed on its ventral surface by the entoderm, whose cells are here and there distended with yolk-spheres.

As shown in the text-figure, the primitive knot ends behind the blastopore in a posterior or "ventral" lip. It is this portion of the knot which is in direct relation with the anterior extremity of the primitive streak; but the cell-mass of the knot is nevertheless histologically distinguishable from the tissue of the fore end of the streak, which it abuts upon.

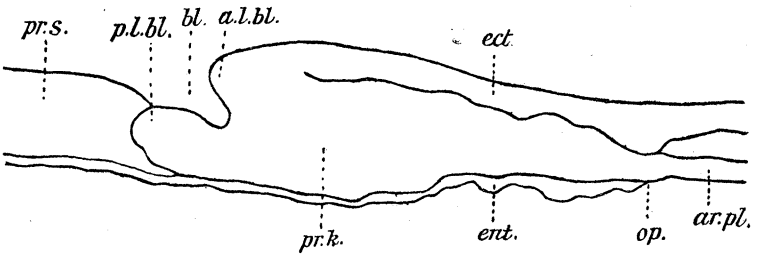
Inasmuch as the relation of the primitive streak to the posterior boundary of the primitive knot is important, in view of the condition of complete independence of these two structures which we have shown to exist in the preceding stage of development, we consider it desirable to offer detailed illustration of the condition just described.

Figs. 29–33, Plate 7, represent photomicrographs of five successive sections through the region extending from the blastopore to the extreme posterior limit of

the primitive knot. In fig. 29 the projecting posterior extremity of the anterior lip of the blastopore appears in the section. Beneath it the curved plate-like mass of the knot is clearly defined. In fig. 30 the anterior lip has disappeared from the plane of section, whilst in fig. 31 the remainder of the knot is obviously narrowing into its posterior lip. In the next section, fig. 32, the much narrowed posterior lip is seen, clearly defined on either side from the adjoining ectoderm in which it is intercalated. In fig. 33, from the succeeding section, the knot tissue has practically disappeared, though the presence of two or three cells may still indicate the last trace of its existence. Behind this plane the definitive ectoderm extends across the median plane with at first only very little, if any, trace of actual primitive-streak structure; though its deep surface is, in the median plane, not quite independent of the underlying mesoderm.

From the figures it will be evident that this posterior part of the primitive knot constitutes a plate-like mass, resembling the "primitive plate" of WILL's writings.

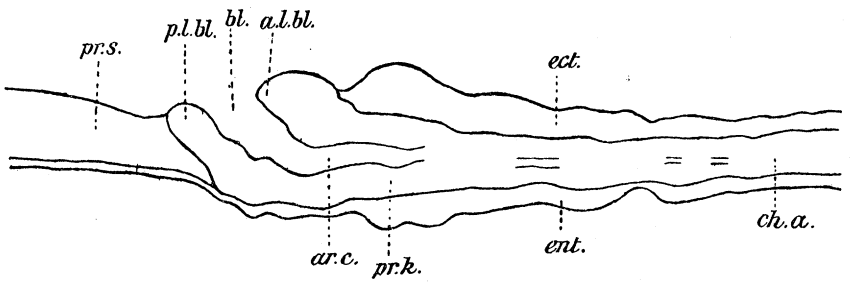
The other specimens of this stage show essentially similar features as regards the posterior limitation of the primitive knot and its relation to the anterior end of the primitive streak. Specimen E is probably the most advanced example of the stage. It differs from Specimen P so far as the posterior portion of the knot is concerned, only in the somewhat increased thickness of the plate-like mass; and this increase in thickness is shared by the ectoderm in the neighbourhood, and by the primitive streak as a whole. Text-fig. 11 represents a schematic mesial-plane-reconstruc-



TEXT-FIG. 11.—Schematic Mesial Plane-reconstruction of Archenteric (HENSEN'S) Knot and adjacent Regions in Specimen E. Reference letters and magnification as in text-fig. 10.

tion from the serial sections of this specimen, drawn at the same magnification and in the same manner as that of text-fig. 10. Comparison with the latter reveals the fundamental agreement of organisation in the two cases. We possess a parallel series of photomicrographs to those in figs. 29–33, but from motives of economy we reproduce only one (fig. 34, Plate 7), taken a short distance behind the mouth of the blastopore, and illustrating the quasi-independence of the knot-tissue from its environment. At the extreme posterior limit it is less easy than in Specimen P

to differentiate the cellular mass of the knot from the cellular tissue of the anterior end of the primitive streak. Nevertheless, the posterior contour of the knot visible in the schematic text-fig. 11, is not merely conjectural, though it is less distinct than in Specimen P. The distinction, in fact, persists until a still later stage of development. Text-fig. 12 represents a mesial-plane-reconstruction similar to



TEXT-FIG. 12.—Schematic Mesial Plane-reconstruction of Archenteric (HENSEN'S) Knot and adjacent Regions in Specimen H.

ch. a., chorda-Anlage; other reference letters and magnification as in text-fig. 10.

those of text-figs. 10 and 11, from the serial sections of Specimen H belonging to a considerably later stage of development (*cf.* figs. 76 and 77, Plate 1). In this the same differentiation, of the posterior boundary of the knot from the anterior end of the primitive streak, is still apparent. Fig. 35, Plate 8, represents a photo-micrograph of a section across the posterior lip of the blastopore from this last specimen, showing a quite definite limitation of the knot from its environment.

It will appear from the inspection of the text-figs. 10–12 that, in front of the blastopore, the accession of the dorsal lip adds greatly to the thickness of the primitive knot. In this situation the knot forms a slight elevation above the general level of the surface of the blastoderm; whilst its ventral or deep surface, clothed by the entoderm, comes more and more to project as a prominent bulging mass into the interior of the vesicle, as we trace it forwards.

For a short distance (about 0·06 millim.) immediately in front of the blastoporic aperture, the undifferentiated cellular tissue of the primitive knot projects directly on the surface. The differentiated ectoderm on either side of it simply merges in the superficial portion of the indifferent tissue of the knot. In front of this region, however, the differentiation of the ectoderm begins to extend medially over the knot, so that the superficial cells of the latter become delimited as a definite ectodermal stratum. This soon comes to extend completely across the median plane, as is indicated in text-fig. 10 by the appearance of the deep contour-line of the ectoderm. This point, then, may be regarded as the anterior limit of the undifferentiated tissue of the anterior lip of the blastopore.

In front of this, the deeper undifferentiated remainder of the knot-tissue constitutes the parietes of the archenteric canal.

These structural characters are illustrated by the series of figures (36–39, Plate 8), which are reproductions of photomicrographs of sections of the knot in Specimen P. Fig. 36 represents the section immediately in front of that shown in fig. 29; and figs. 37 and 38 the third and fourth sections, respectively, in front of the same section. Figs. 39 and 40 represent the third and fourth sections, respectively, in front of the plane of fig. 38, *i.e.*, about 0·07–8 millim. in front of the blastoporic aperture. In the last of these sections the ectoderm has at last become completely established as a definite layer over the surface of the mass of the knot, in whose interior a mere vestige of the archenteric cavity is visible.

From the sections of Specimen P we prepared a wax-plate reconstruction-model of the primitive knot and its immediate environment, at a magnification of 400 diameters. The superficial aspect of this model displayed the blastoporic aperture as an asymmetrical cleft-like aperture of relatively considerable width (it measured over 60 millims. in the model). This cleft was not transverse, but somewhat oblique, and bore traces of an original crescentic disposition convex posteriorly, though the overhanging dorsal lip which actually defines it is not now even, but irregular in its contour. This dorsal lip projects backwards, and it is its free margin which is seen shaved off in fig. 29. From this latter figure, together with figs. 36 and 37, the asymmetry of the blastoporic aperture may be realised, as also the fact that it is the apparent right (actual left) horn of the crescentic opening which is prolonged deeply to form the commencement of the imperfect blastoporic canal. The other horn and the remainder of the crescentic aperture form little more than a deep groove continuous with the canal at its mouth. In the slightly older Specimen E the crescentic character of the aperture has entirely disappeared: the opening is still somewhat asymmetrical, but appears as a somewhat irregular, single, and approximately medial, depression, though its vestibule is wide and bears faint traces of its original character (*cf.* fig. 34).* This wide dorsal opening leads into a blind depression (*cf.* text-fig. 11, p. 74), representing the lumen of the archenteron. Fig. 41, Plate 9, shows a photomicrograph of a section through the primitive knot of Specimen E immediately in front of the apparent blind ending of the blastoporic canal. Here, again, the ectoderm is seen in process of differentiation over the dorso-lateral surfaces of the knot, as if by a method of overgrowth. It is, however, at least in the main, a process of differentiation *in situ*. In the next section figured (fig. 42), 0·03 millim. in front of the last, partially differentiated knot-tissue projects like a plug mesio-dorsally; but careful examination shows that this mesio-dorsal mass is not becoming overgrown by, but is actually becoming transformed into, definitive ectoderm. The next figure (fig. 43) 0·024 millim. further on, illustrates the completion of this differentiative process.

* Asymmetry of the dorsal blastoporic opening would seem to be a very common character in mammals, and we are of opinion that this asymmetry is referable to the primitive and original crescentic character of the opening and the longer persistence of one horn of the crescent.

(e) *The Archenteron and its Walls in the Postgastrular Stage.*—We have already shown in this and in our earlier communication ('03) that in the preceding "gastrular" stage the archenteric cavity is not a tubular canal, but is relatively wide and dorso-ventrally flattened. In these respects it agrees with the corresponding stage of the reptilian archenteron as represented in the descriptions and figures of various authors, *e.g.*, MEHNERT, WILL, MITSUKURI, etc.

But even in the "postgastrular" stage now under consideration it is easy to obtain the clearest evidence of the fact that the typical and original form of the archenteric canal is that of a transversely elongated and dorso-ventrally compressed cavity. It is true that it no longer possesses this character throughout. We have just seen that the blastoporic aperture bears traces of its primitive widely extended form. In front of this, however, we have seen that the canal undergoes interruption, amounting in some places to complete suppression and obliteration. This is most marked in the slightly older Specimen E, as illustrated in figs. 41–43. But even in Specimen P the partial obliteration of the canal is illustrated in the series of figs. 36–39 (*cf.* also text-figs. 10 and 11). We are inclined to attribute this more or less complete obliteration to the occurrence of a somewhat energetic cell-proliferation in the parietes of the canal. In any case, this obliterative phase is not necessarily a permanent one, for at a still later stage (*cf.* text-fig. 12, from Specimen H) we find that a very distinct canal reappears in the same position.

But it is in the more anterior region of the primitive knot in the postgastrular stage that we obtain the clearest evidence of the original form of the archenteric lumen.

Fig. 44, Plate 9, represents a section across the primitive knot in Specimen P, in a plane 0·15 millim. in front of the plane represented in fig. 40. Here the knot appears as somewhat wider and more flattened than it is behind this plane. A very evident lumen is present in the form of a transverse cleft separating ventral and dorsal walls, or roof and floor, of the archenteron. A close resemblance may be observed between fig. 44 and figures of corresponding sections through the archenteron in reptiles, as, for example, figs. 444 and 445, in HERTWIG'S 'Handbuch' ('03).

As we pass backwards from the plane represented in fig. 44, we find that the lumen undergoes increasing interruption in the way of fusion and adhesion of its dorsal and ventral walls, so that the lumen becomes broken up into a number of separate cavities. In one specimen we have an example of a row of such cavities replacing the continuous transverse fissure. More commonly, however, only two or three small cavities persist; and there is an evident tendency towards the retention of these remains of the lumen in certain more or less definite situations, viz., in positions corresponding to the lateral extremities of the original fissure, and, less commonly, in a medial position. Even in fig. 44 the lumen is not wholly

free from interruption of the kind referred to, and thus there appears, on the right, a small isolated cavity which represents the original lateral portion of the lumen.

Further back, nearer the blastopore, in the region illustrated in figs. 37–40, we have already stated that irregular remains of the lumen appear in the sections, in situations varying from section to section. As a rule, however, they are met with either on the one side or the other of the median plane, and are to be associated with the prolongation of one or the other of the horns of the crescentic, transversely extended, blastoporic aperture. As we trace the sections forwards, then, we find that these small cavities, representing portions of the original lumen, appear, disappear, and reappear in corresponding positions either on the same, or on opposite, sides, and occasionally on both.

We have already called attention, in connection with text-fig. 3, to the occurrence of a median interruption of the cavity of the wide archenteron in the earlier, gastrular, stage, at a short distance in front of the blastoporic aperture. It is quite likely that this represents the commencement of the process of proliferative coalescence of the walls of the archenteron which leads to the condition above described. It is nevertheless true that here and there a median vestige of the lumen may be discerned, so that the coalescence in question cannot be uniformly median and symmetrical.

Further, one vestigial space is occasionally found more or less dorsally placed with respect to another. In such sections the arrangement of the cell-groups around the spaces points to the occurrence of a crumpling of the walls of the originally simple transverse lumen; and abundant evidence of the actual occurrence of such a process is obtained in slightly more advanced specimens.

From the above description it will appear that, as the serial sections are traced forwards, in Specimen P, from the region of the anterior lip of the blastopore, the vestiges of the archenteric cavity become more numerous; and these presently coalesce with one another to constitute the wide transverse cleft seen in fig. 44, which represents the original, and practically unmodified, archenteric lumen.

The simple and primitive character represented in fig. 44 is only manifested for a very short distance. In the next section but one in front of the plane of fig. 44, disintegration of the ventral wall of the archenteron sets in, resulting in the disappearance, within a few sections, of practically the entire floor of the cavity. This process is well illustrated in the series of figures (45–48), representing photo-micrographs of four consecutive sections from this region.

The result of this process of disintegration of the ventral wall of the archenteron is, that, at a distance of just under 0·3 millim. in front of the blastopore, the archenteron is represented solely by the forward continuation of its dorsal wall or roof, constituting a flattened "archenteric plate." So long as the walls of the

archenteron are complete, forming the mass of the "primitive knot," its ventral surface is clothed by entoderm. But the disintegration of the ventral wall is accompanied by a disappearance of the underlying entoderm, though fragmentary remains of the latter may persist for a short distance forwards. When the denudation of the dorsal wall of the archenteron is complete, the latter is intercalated, as an "archenteric plate," in the roof of the general cavity of the blastodermic vesicle, into which the lumen of the archenteric canal now opens, ventrally.

Fig. 49, Plate 3, represents a drawing after a photograph of the wax-model referred to on p. 76. The figure represents the ventral aspect of the model, in the construction of which the existence of an entodermal sheet was ignored. The marginal portions of this surface of the model accordingly represent the ventral surface of the mesodermal sheet. Rising above the general surface of the mesoderm is seen the smooth prominence of the ventral wall of the primitive or archenteric knot. Posteriorly this gradually slopes away towards the hinder edge of the model, which just includes the anterior extremity of the primitive streak. The tissue of the latter is in no way distinguishable from that of the mesoderm on either side of it. Anteriorly, the ventral wall of the archenteron is deficient. The irregular free anterior margin of its posterior persistent portion, *i.e.*, the primitive knot, stands out abruptly. In front of the latter the denuded and exposed ventral surface of the persistent roof of the archenteron, constituting the archenteric plate, is clearly seen. It is to be remembered that this plate extends forwards with little alteration, save slight variation in width, into the anterior region of the embryonic area, where it finally widens out into a so-called "protochordal" expansion. A representation of the plate in its entirety in the model would thus demand the continuation forwards of the latter for a distance of no less than 65 centims. beyond its present limit.

Both from the model and from figs. 47 and 48, it is apparent that the ventral surface of the archenteric plate is at first somewhat rugged and irregular. Indeed, it is evident that here and there clumps of cells originally belonging to the ventral wall of the archenteron have escaped disintegration, remaining adherent to the surface of the archenteric plate. Such isolated masses appear in the model (fig. 49) as well as in fig. 50, which represents a section across the archenteric plate in the plane of the most anterior of the cell-clumps of this character shown in the figure of the wax-model. From this sectional figure it will also appear that the preservation of such isolated and fragmentary remains of the original ventral wall of the archenteron may be associated with the preservation of localised patches of yolk-entoderm as well.

The figure of the model may also be compared with advantage with the schematic text-fig. 10, constructed from the same sections, though reproduced at a somewhat different scale of magnification.

As the archenteric plate is followed, in the serial sections, in an anterior (cephalic) direction, it gradually loses its rugged character, becoming flat and even. It also

diminishes in thickness. In the region of the intact archenteron, or primitive knot, both the roof and the floor of the gastrulation-cavity consist of several layers of cells. After disappearance of the ventral wall in the manner above described, the somewhat irregular dorsal wall at first retains its thickness. Further forwards it becomes two-layered, and finally, still more anteriorly, it comes to consist of only a single layer of cells. When this condition is attained its free surface is seen to be not only smooth and even, but sharply defined, as is shown in the photomicrograph reproduced in fig. 51, Plate 10.

In all its essential features the archenteron in Specimen PP showed great similarity to that of the Twin-specimen P. As in the latter instance, the intact portion of the archenteron appeared, on surface-view, as a "primitive knot," similar in form and appearance to that found in Specimen P. There was a similar blastoporic aperture leading into the interior of the massive "primitive knot." But the archenteric cavity was more imperfect than that of the twin-specimen. It was represented by minute clefts or partial tubular canals, as in the posterior part of the knot in Specimen P. Nowhere were these found to coalesce so as to constitute a wide cleft-like lumen. Nevertheless, the spaces occupied a plane corresponding to that of the lumen in Specimen P, and obviously constitute a virtual transverse archenteric cavity.

Further, the ventral portion of the massive archenteron, forming the floor of its virtual cavity, undergoes disintegration, and disappears, exactly as happens in Specimen P, except that the caudal limit of denudation is, in this case, slightly nearer to the plane of the blastopore, viz., about 0·2 millim. in front of the latter instead of nearly 0·3 millim. as in the twin-specimen.

As in Specimen P, so also in PP, the primitive knot, immediately in front of the blastopore, is of considerable dorso-ventral extent, but decreases in thickness as it is traced forwards. This decrease in thickness is not, as in the previous specimen, accompanied by a simple arrangement of the cells around a wide transverse archenteric cavity (as shown in fig. 44). In the specimen now under consideration the cells do undergo some rearrangement, but into the form of groups or masses. The constituent cells of each mass tend towards a disposition about a central cavity, actual or potential; and each focal cavity obviously represents a segregated portion of the original cleft-like archenteric cavity.

The differentiation of these cell-groups is, however, confined to a very limited region immediately behind the plane at which the ventral archenteric wall undergoes disintegration. But for the fact that they attain to great definiteness and extension in the slightly more advanced Specimen E, they might have been passed over without special remark in the present case. Figs. 52–54 represent photomicrographs of three consecutive sections across the archenteron in Specimen PP, the last of which shows the disintegration of the archenteric floor. It is easy to see, if a comparison be made between figs. 44 and 45 on the one hand, and figs. 52–54 on the other, that

the continuity of the archenteric lumen, illustrated in the former figures, has been interrupted in the latter by localised fusions of the dorsal and ventral walls, which interruptions appear as if they resulted from a series of indentations or inflexions of the walls of a primitively simple cavity. In any case the structural result, as illustrated in figs. 52 and 53, is the differentiation of cell-masses, consisting of cell-elements belonging morphologically to both the ventral and the dorsal walls of the archenteron. In the next succeeding section, illustrated in fig. 54, the disappearance of the ventral cell-elements of these masses is seen in progress.

It will be shown later, in connection with the examination of Specimen E, that the arrangement of the cell-masses here referred to is probably not a wholly fortuitous one. Even here there is a definite suggestion of a bilaterally symmetrical arrangement. The lumina, actual or potential, of the lateral cell-masses, would seem to represent the lateral angles of the primitive wide archenteric lumen. And although in Specimen P such masses cannot be said to appear, yet in fig. 44 the small isolated cavity to the right of the main archenteric lumen is in all probability homologous with the lumina of the laterally placed cell-masses which appear in Specimen PP.

We have found the histological study of the sections of Specimen PP, in the region of the ventral archenteric defect, capable of throwing light upon the nature of the process of disappearance of the archenteric floor. The series of figures (44–48) has already demonstrated the abrupt disappearance of considerable groups of cells. Nevertheless we have ascertained, more especially in the case of the Twin-specimen PP, that the disappearance of these cells must not be regarded as simply a dropping-out, or breaking-away, of portions of the archenteric floor, *en masse*.

It has already been stated that the disappearance of the ventral wall of the archenteron involves the destruction of the portion of the entodermal sheet underlying it, so that the archenteric plate comes to be in immediate relation with the cavity of the blastodermic vesicle. Yet it is evident from figs. 45–47 that the entoderm may persist, not merely fragmentarily, but to some extent as a layer, even beneath considerable gaps in the archenteric floor. Even in fig. 48, in which practically the entire floor of the archenteron has disappeared, the underlying entoderm is still represented by yolk-entodermal cells, partially degenerate, and held in their natural position in the section by the celloidin-infiltration which preceded the paraffin-embedding.

It is, however, only for a few sections that the integrity of the entoderm is preserved, even to the extent shown in the figures referred to. Further in front, where the archenteric plate has been definitely established, the entoderm underlying the archenteron is represented only by isolated cells which are found here and there adherent, either directly to the exposed ventral surface of the archenteric plate or to a reticulum covering this surface.

It is easy to understand the persistence of entodermal cells where these are found

clinging to clumps of cells forming obvious remnants of the ventral wall of the archenteron, as seen in fig. 50, and again in fig. 55, from Specimen PP. It is less easy to account for the presence of entodermal cells directly attached to the denuded and exposed surface of the archenteric plate. Such a condition seems to be witnessed in figs. 56 and 57. It must be admitted, however, that in such instances the entodermal cells are not very intimately attached to the surface of the plate, and are not infrequently separated from it by a layer of the reticulum already referred to. This reticulum is especially apparent in the last two figures quoted. An answer to the question, What is the nature of this reticulum? may, in our opinion, be arrived at by an examination of the series of sections illustrated in figs. 54–57.

Fig. 54 represents a section in which a mass of cells forming part of the ventral wall of the archenteron is still preserved intact. The persistence, in this and in other sections, of masses or small clumps of cells originally forming part of the ventral parietes of the archenteric cavity, is in our opinion to be explained as due to the previous occurrence of localised adhesions between ventral and dorsal walls of the archenteron, causing such partial obliterations of the lumen as we have already noted in this specimen, behind the plane of the archenteric defect. When disintegration of the archenteric floor sets in, those portions of it which are more intimately adherent to the dorsal wall longest resist denudation, and accordingly remain as islands of cells forming projections from the surface of the archenteric plate.

A non-denuded area of this character is seen in fig. 54. On either side of it are gaps representing regions in which the cellular floor of the (here largely virtual) archenteric cavity has undergone, or is undergoing, disintegration. These gaps are partly occupied by a reticular formation in which are still embedded a few nuclei. The reticulum itself represents the remains of the bodies of those cells of the archenteric floor which are in process of disintegration. This interpretation has been confirmed by examination under the oil-immersion lens, by whose aid the contours of the degenerated cells could in some cases be observed. The nuclei in the reticulum occupying the gap on the left, in the section, are simply the nuclei of such degenerate cells of the archenteric floor; whilst, ventral to these again, and to the gap which they occupy, are the nucleated bodies of several yolk-laden cells of the entoderm which originally clothed the ventral surface of the intact archenteron

It appears quite certain, from the facts and observations above set forth, that the disappearance of the ventral wall of the archenteron in the blastoderm of Ornithorhynchus takes place primarily through an intracellular degenerative process, and is not to be represented as one of mere mechanical disruption. The interpretation given above fully explains the retention of entodermal cells here and there over the otherwise bared surface of the archenteric plate. That retention is due to adhesion through a reticulum of more or less degenerate cell-material. As might be expected, such vestiges are most abundant in the vicinity of the posterior limit of the archenteric plate, where the process of disintegration is still in operation.

It has already been stated that the archenteric plate immediately in front of the archenteric deficiency (ventral aperture of the blastoporic canal) is composed of more than one layer of cells, and that it becomes gradually reduced to one layer as it is traced forwards. At the same time, it loses its ragged appearance and becomes smooth and sharply defined. It is possible that this reduction may also be brought about by degeneration of the more superficial cell-layers. This is at least suggested by the consideration of the photomicrograph in fig. 57.

The significance of the extremely elongated forward extension of the archenteric plate will be discussed in a subsequent section of the paper.

In the meantime we have to direct attention to certain structural features of the differentiation of the archenteron, in the way of distinction between its medial and its lateral regions, which are recognisable in this postgastrular stage. And, inasmuch as this differentiation is closely bound up with that of the mesoderm in its vicinity, it will be found advantageous first to consider the question of the relation of the mesoderm to the axial structures in the embryonic area, at this period of development.

(f) *The Mesoderm and its Relation to the Structures of the Embryonic Axis.*—In the section of this paper dealing with the "gastrular" stage, it was shown that a tolerably extensive sheet of mesoderm had already been produced from the linear area of proliferation of the primitive streak, at a time when the "primitive knot," though it exhibited a gastrulation-cavity, had as yet given origin to no mesoderm whatever. Indeed the archenteron (*i.e.*, the primitive knot and its gastrular cavity) is, during that period, wholly unconnected with, and remote from, the sheet of mesoderm originating from the primitive streak (so-called "peristomial" mesoderm) (*cf.* text-fig. 7, p. 55).

Now, in the postgastrular stage, as exemplified in our Specimens P and PP, the sheet of mesoderm arising from the primitive streak has extended over a considerably wider area. Most notably it has spread forwards, on either side of the primitive knot and of its anterior continuation, the archenteric plate, so as almost to reach the anterior limit of the pellucid or embryonic area. We have already seen that, at the hinder margin of the primitive knot, the primitive-streak tissue is in quasi-continuity with the archenteric tissue. The examination of figs. 38–40 will prove that, at the lateral margins of the archenteric knot, there is now genuine continuity between the latter and the neighbouring mesoderm. Cells are now evidently being added to the mesoderm by proliferation from the lateral archenteric margins. Occasional mitoses are met with in the marginal cells of the knot, but the contribution thus far obtained is still relatively inconsiderable. And, when we pass further forwards into the region of the archenteric plate, we find (figs. 50–51) that, gradually, this proliferative connection becomes lost, and that the medial margin of the mesodermal sheet merely lies in contact with, and slightly overlaps, the lateral margin of the plate (fig. 51). It may also here be remarked that, with the disappear-

ance of the subarchenteric entoderm, the entodermal sheet on either side acquires a connection with the edges of the archenteric plate, as is shown in the figures cited.

When the mesodermal sheet, on either side, in front of the region of the primitive knot, is examined in transverse sections, it is found to be distinguishable into two regions. The one coincides with that broad "paraxial zone," already described in connection with the surface-description as approximately coterminous, laterally, with the area of thickened ectoderm. When this broad band of paraxial mesoderm is followed outwards, in the transverse sections, from its medial edge, it is found to undergo some thickening, though of no very marked character. This is maintained as far as the peripheral region of the thickened ectoderm. As a whole, however, it is so insignificant in thickness, so ill-defined, and so wide in its lateral extension, that at first one hesitates in recognising in it the zone of approaching protovertebral segmentation. Comparison with subsequent stages shows, nevertheless, quite conclusively, that the whole of this feebly indicated paraxial zone of mesoderm does, in fact, correspond to the region occupied by the future somites. Possible indications of commencing protovertebral segmentation have been alluded to in connection with the photomicrographs in figs. 22 and 23.

It is further to be clearly recognised that the "paraxial zone" of mesoderm, as above defined, is nothing more than a somewhat thickened zone of that alar extension of mesoderm which has grown forwards, on either side of the primitive knot and its archenteric prolongation, from the mesodermal sheet occupying the "primitive-streak area" in the preceding gastrular stage. It will presently be shown that its medial edge is reinforced by the accession of cellular material derived from the archenteron.

It may be noted in passing that the very trifling predominance in thickness of the paraxial zone of mesoderm is by no means confined to monotremes. The considerable thickening of paraxial mesoderm, which in the typical vertebrate heralds the appearance of protovertebral segmentation, is in all probability conditioned by the elevation of the medullary folds. Accordingly we find that, in cases in which the medullary plate remains for a relatively long period spread out on the flat, without the dorsal development of medullary folds, the paraxial mesoderm is not distinguished by any very conspicuous thickening. Even when quite definite somites have been differentiated, they remain thin plates, and do not appear, in the transverse sections, as the obvious thick masses, so familiar in the case of chick development, and in probably the majority of mammals. From the figures in SELENKA'S memoir on the development of the Opossum ('86–7) it is evident that in that form the paraxial mesoderm shows the same characteristics as those described above in Ornithorhynchus; and other marsupials examined by ourselves likewise conform to the monotreme type in this respect.

The other, more outlying, "parietal zone" of mesoderm extends outwards beyond the paraxial mesoderm, in the peripheral region of the embryonic area, and into the area opaca.

The characteristics of the mesodermal sheet, as seen in sections which cut across the archenteric plate, are diagrammatically illustrated in the text-fig. 9, to which reference has already been made.

It will be noticed that the inner or medial portion of the paraxial mesoderm thins as it is traced towards the archenteric plate, and, in Specimen P, when it reaches the neighbourhood of the plate it has become reduced to practically a single layer of somewhat flattened cells. The inner margin of this sheet projects into the angle between the ectoderm and the archenteric plate, slightly overlapping the latter and discontinuous with it, as has already been pointed out (fig. 51). On the other hand, as has also been indicated, the mesodermal sheet in the region of the primitive knot, or intact archenteron, is actually in proliferative continuity with the edges of the latter, so that there, genuine "gastral" mesoderm is being produced.

But this trifling accession of gastral mesoderm does not represent the whole contribution made by the archenteron to the embryonic mesoderm. Figs. 56 and 57, Plate 11, represent two sections across the archenteric plate in Specimen PP, a short distance in front of the plane at which the floor of the archenteron becomes deficient. In both of these a differentiation is recognisable between medial and lateral portions of the plate. The medial or axial portion of the plate includes about three-fifths of the width of the plate, and is gently concave in a ventral direction. The lateral marginal portions are slightly reflexed in a dorsal direction. The ectoderm overlying the plate forms a somewhat thickened plaque, slightly thicker where it overlies the junction between the axial and lateral parts of the plate. The differentiation of the marginal parts of the archenteric plate is not due merely to their dorsal flexure, but partly also to actual demarcation of their constituent cells. This is especially well seen in fig. 56. Now, when these marginal portions of the plate are traced in a caudal direction through the serial sections, they are found to merge in the lateral cell-masses which are constituted by the lateral parietes of the intact archenteron, as shown in fig. 53 (*cf.* also the intermediate figs. 54 and 55). These lateral cell-masses of the primitive knot are connected at their margins with the thin mesoderm on either side, but they are at present only in slender proliferative connection with it. On account of the occlusion of the lateral portions of the archenteric lumen which primitively penetrate them (*cf.* fig. 44), it is somewhat difficult to determine to what extent they share in the disintegration of the archenteric floor, and suffer denudation of their ventral cell-elements. But their dorsal portions, at least, are directly continued forwards into the marginal portions of the archenteric plate, seen in figs. 56 and 57.

When the archenteric plate is traced a small fraction of a millimetre further in a cranial direction, the visible differentiation of medial and lateral portions entirely disappears; so that a condition is arrived at similar to that shown in fig. 51 from the twin-specimen and in the schematic text-fig. 9. In respect of the

differentiation of cell-masses in the archenteron, and of marginal portions of the archenteric plate, Specimen PP must be regarded as slightly in advance of the sister specimen.

It will appear in the sequel that those lateral derivatives of the archenteron are destined to become converted, *en bloc*, into mesoderm which coalesces with the inner edge of the already existing mesodermal sheet, so that in them we have a further contribution of " gastral " mesoderm.

(g) *The Archenteric or Gastral Mesoderm in Specimen E.*—The genesis and the destiny of the direct mesodermal derivatives of the archenteron in the postgastrular stage may be followed with advantage in connection with the primitive knot and archenteric plate of Specimen E, which is probably the most advanced example of the stage.

The general condition of the archenteron in this specimen has already been illustrated schematically in text-fig. 11, from which its general resemblance to Specimen P, as illustrated in text-fig. 10, is clearly manifest. The most anterior portion of the embryonic area was lost in preparation of the blastodermic membrane, and it is therefore impossible to estimate the total length of the archenteron. In several respects a slightly more advanced condition of this specimen is evidenced. Thus, whilst the antero-posterior extent of the primitive knot or intact archenteron has remained practically the same, the transition from it to the ventrally denuded archenteric plate in front, is of a less abrupt character than in either of the two previously described specimens, P and PP. This is due to a further progress in the differentiation of the tissue of the intact portion of the archenteron behind the plane of continuity with the archenteric plate. A rearrangement of this into cell-masses, similar to those present in a few sections just behind this plane in Specimen PP, has now taken place throughout a considerable extent of the intact archenteron, or primitive knot. This continued differentiation of cell-masses from the tissue of the parietes of the intact archenteron is accompanied by a progressive diminution in their thickness as they are traced forwards, so that they now merge more gradually into the archenteric plate which represents the denuded dorsal wall of the original archenteric cavity. As was the case for a short distance in Specimen PP, the originally transversely elongated lumen of the archenteron (as recognisable in figs. 44–46, from Specimen P) has been broken up, and largely obliterated, by the process of rearrangement of the cells of the walls of the original cavity involved in the differentiation of the cell-masses referred to. The differentiation in question is still absent from the portion of the primitive knot which lies immediately in front of the blastopore. The canal or depression leading from the latter into the archenteric knot appears to end blindly, although small spaces exist in front of its apparent termination which are vestiges of the original lumen.

In front of the primitive knot the elongated archenteric plate shows, in a more pronounced fashion, and far more extensively, a differentiation into medial and lateral

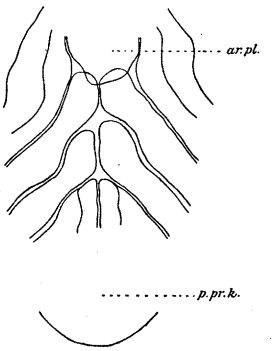
regions comparable to that which was evident in the slightly earlier Specimen PP. And here also the lateral portions of the archenteric plate appear to be in series with the lateral cell-masses differentiated from the archenteric tissue of the primitive knot. But the differentiation of these lateral portions from the median region of the plate has now progressed so far that they may be readily recognised as laterally-placed collections of cells, more or less distinct from the remainder of the plate, and as having entered into an intimate connection with the formerly free and attenuated medial edge of the neighbouring paraxial mesoderm.

The general outline here given of the structural characters of the archenteron and its derivatives in Specimen E, may now be supplemented, and illustrated in detail, with the aid of photomicrographs of a number of the transverse sections.

Fig. 58, Plate 11, represents a section across the archenteron, about 0·15 millim. in front of the blastopore. This section shows traces of the original archenteric lumen, whilst it also shows indications of a commencing rearrangement of the constituent cells into masses. From this point forwards such a rearrangement becomes more and more distinct. Figs. 59–63 represent sections taken at intervals throughout a distance of just under 0·2 millim. in front of the plane of fig. 58, and illustrating, from behind forwards, in that region, the appearance of the cell-masses referred to. From these figures it will be evident that the appearance of the masses undergoes some change from point to point. They tend to appear in bilateral, though rather asymmetrical, pairs; usually two or three pairs, though in some sections (unfigured) only one, being more or less distinctly recognisable in a section. Lumina, actual or potential, are commonly found in the interior of the masses, though this is not the case in every section.

Consideration alone of the sections figured would tend to give rise to the impression that the masses shown in section were of the nature of partially canalised, more or less cylindroidal masses, running parallel to the median plane. When, however, the complete series of sections through the region illustrated in figs. 58–63 is examined, it is easy to see that the masses do not run longitudinally, but obliquely, with reference to the median plane. The cell-masses which lie close to the median plane in the anterior sections of the region are not the same masses as those which are found close to the median plane further back. The former, when they are followed backwards, are found to diverge gradually from the middle line, thus making room for new masses which appear on either side of, and close to, the median line, and which in their turn diverge as they are followed still further in a caudal direction. In text-fig. 13 we reproduce a schematic plane surface-projection of the masses in question, constructed from the serial sections. The figure gives a correct idea of the general plan of arrangement, whilst it somewhat exaggerates the degree of regularity which prevails. It is obvious that what we have presented is a bilateral series of segments of archenteric tissue, limited by more or less parallel and oblique lines of segmentation. These limiting lines are constituted by the constrictions of the archenteric tissue which

are visible in the transverse sections figured, where they form the boundaries of the several cell-masses. When traced in a caudo-lateral direction, each of the oblique segments, with the exception of the posterior, thins out, and merges in the



TEXT-FIG. 13.—Schematic Horizontal Reconstruction of the " protosomites," or segmental cell-masses, in the Archenteric Knot of Specimen E.

ar.pl., archenteric plate; *p.pr.k.*, posterior undifferentiated portion of the Archenteric Knot. Magnif. = × 100.

unsegmented sheet of mesoderm, which, as already stated, is in this stage definitely continuous with the lateral margins of the archenteron. On the other hand, the most caudally placed members of the series are distinct only in their mesio-anterior portions and are lost posteriorly in the less differentiated tissue of the caudal part of the archenteric knot.

On account of the more or less symmetrical oblique segmentation thus displayed throughout a considerable extent of the intact archenteron or primitive knot, we venture to designate the cell-masses or segments above described as " protosomites." It is to be clearly understood that the term suggested is not to be taken as implying that these segments represent a primitive stage in the differentiation of the definitive somites, for this they certainly do not. But it seems justifiable to regard them as in some way representing a metameric segmentation occurring in the walls of the primitive archenteron. As indicated in text-fig. 13, we have been able to recognise five pairs of these "protosomites" in the anterior region of the intact portion of the archenteron.

It has been pointed out that the transition from archenteric knot to archenteric plate is much less abrupt and sharply defined in the specimen now under consideration than in the other specimens belonging to the same postgastrular stage. In fig. 60, Plate 12, the layer of entoderm underlying the now much flattened archenteric knot is quite intact; and it is still present, though incomplete, in the two consecutive sections shown in figs. 61 and 62 slightly in front of the plane represented in fig. 60. On the other hand, in fig. 63 (three sections in front of the plane of fig. 62), not only has the axial portion of the entodermal sheet entirely

disappeared, but disintegration and loss of the ventral archenteric tissue has already set in.

At the plane represented in fig. 61 three pairs of protosomites are distinguishable in the knot, definitely on one side and more vaguely on the other. When the plane of fig. 63 is reached, we find that the ventral portions of the more medial protosomitic masses are being lost, and that their dorsal portions, becoming confluent across the medial plane, now constitute the posterior extremity of the archenteric plate. This is well seen in fig. 64, representing the second section in front of the plane of fig. 63. In this figure the narrow axial archenteric plate is sharply contrasted with the thick laterally placed protosomites, which have not yet suffered loss of their ventral portions. The latter, indeed, still show small but distinct lumina—vestiges of the original archenteric cavity.

As the archenteric plate is traced forwards it is found to widen at the expense of the next more laterally placed pair of protosomites. These also become thinned out by ventral disintegration, and their denuded dorsal portions become incorporated with the margins of the archenteric plate, which thus becomes rather abruptly widened. This step is illustrated by the two consecutive sections shown in figs. 65 and 66, the former of which is 0·03 millim. in front of the plane of fig. 64.

Fig. 67 represents a section 0·08 millim. in front of fig. 66. It shows a further degree of widening of the archenteric plate, which has, indeed, now reached its maximum width—a width which practically coincides with that of the entire archenteron further back. But this width is not maintained without variation as the plate is followed in an anterior direction. At frequently recurring, though not quite regular, serial intervals, the plate undergoes constriction in width. This intermittent constriction is associated with, and is doubtless directly dependent upon, the emancipation of marginal portions from the axial region of the plate, and their differentiation as somewhat thickened masses of cells continuous with the sheet of paraxial mesoderm. They thus seem to constitute reinforcements of true gastral mesoderm contributed to the pre-existing mesodermal sheet, which latter, as has been already shown, owes its existence to an original proliferation from the primitive streak. It was pointed out in connection with our examination of the sectional anatomy of specimens P and PP that, whilst the mesodermal sheet was then genuinely continuous with the margins of the primitive knot, its relation to the margins of the archenteric plate was one of contiguity and not of actual continuity. In the present specimen, however, the paraxial mesoderm has become largely continuous with the edges of the archenteric plate, and this continuity appears to be a preliminary to the above-described differentiation of masses of genuine gastral mesoderm from the lateral portions of the archenteric plate. Attention was previously drawn to an early phase of such a differentiation in specimen PP in connection with figs. 56 and 57. In specimen E this process has extended forwards, though in a discontinuous fashion, along the margins of the archenteric plate.

It may here be repeated that the masses of gastral mesoderm thus differentiated are in series with the protosomites or cell-masses which constitute the lateral portions of the intact archenteron further back. These protosomitic cell-masses were seen to exhibit a segmental or serial arrangement. The irregular serial recurrence of differentiations of gastral mesoderm from the lateral margins of the archenteric plate further forwards is, at least, suggestive of a serial repetition of segments homodynamic with the "protosomites" of the intact archenteron itself.

(c) *Summary of Characteristics of Postgastrular Stage.*

(*a*) The egg in the postgastrular stage measures about 12–13 millims. in diameter. The shell and vitelline membrane (or zona) present characters similar to those seen in the preceding stage.

(*b*) The egg constitutes a blastodermic vesicle with fluid contents, containing more or less disseminated yolk-material in suspension.

(*c*) As in the gastrular stage, the vesicle-wall is still for the most part bilaminar; the characters of the two layers are identical with those of the previous stage, outside of the region of embryonic differentiation.

(*d*) Instead of the "primitive-streak area" of the gastrular stage, an "embryonic area" has become established by a remarkable anterior extension of the former, and by its annexation of that region in which the primitive knot was situated in the antecedent stage.

(*e*) The "embryonic area" is indistinguishable from, and is here identified with, an "area pellucida," whilst the extra-embryonic blastoderm constitutes an "area opaca" in virtue of its heavily yolk-laden entoderm.

(*f*) An "embryonic shield" in the usual sense is not properly recognisable, since the thickened and cubical ectoderm is here practically confined to that portion of the embryonic area which corresponds, not to the entire "embryonic shield" of other mammals, but to that region of it only which answers to the "paraxial zone" (Stammzone).

(*g*) The embryonic area is occupied by a sheet of mesoderm which is formed by the further extension of that which occupies the "primitive-streak area" in the preceding stage. It is divisible into paraxial and parietal regions, and the latter extends for some distance into the area opaca.

(*h*) Possible indications of early somitic segmentation were recognised in one of the specimens in the shape of oblique lines crossing the paraxial region in front of the plane of the primitive knot.

(*i*) The Anlage of the vascular area is present as a "protangioblastic zone," in the form of a somewhat horse-shoe-shaped zone of the area opaca. This surrounds the posterior half or more of the embryonic area, lying close to, and concentric with, the inner margin of the area opaca. (Histologically this is characterised by a slight

cellular thickening of the mesoderm, and a locally more intimate connection of the latter with the underlying yolk-entoderm.)

(*k*) A primitive or "HENSEN'S" knot, with a superficial blastoporic aperture, is present. Its posterior border is contiguous with the anterior extremity of the primitive streak, from which, however, it is sharply distinguished.

(*l*) The tissue of the primitive or archenteric knot constitutes the parietes of a blastoporic or archenteric canal whose lumen is flattened dorso-ventrally and generally partly obliterated. The anterior limit of the knot is determined by the occurrence of a complete defect of its ventral wall (which forms the floor of the canal), so that the canal opens freely into the cavity of the vesicle.

(*m*) The disappearance of the ventral wall of the archenteron is found to take place primarily through an intracellular degenerative process and not through any merely mechanical disruption.

(*n*) The tissue of the knot, forming the walls of the intact segment of the archenteric canal, exhibits a tendency to rearrange itself in the form of luminated cell-masses. These also tend to manifest a symmetry of arrangement, both serially and bilaterally. The lateral portions at least of these cell-masses become converted into "gastral" mesoderm.

(*o*) The dorsal wall of the primitive or archenteric knot is directly continued forwards in front of the plane of the ventral archenteric defect as an "archenteric plate" or "head-process" of very considerable length. The anterior extremity of this plate terminates by widening out into a "protochordal-plate" expansion.

(*p*) The median portion of the archenteric plate represents the "chorda-plate" proper: its lateral portions—lying in series with the lateral cell-masses of the knot —are destined to undergo conversion into "gastral" mesoderm.

(*q*) The long primitive streak extends backwards (for a distance of about 5 millims.) from the posterior border of the primitive knot, with which it is in contact, to the posterior margin of the embryonic area. Here it widens out into the so-called "caudal knot," and appears to merge in the edge of the area opaca. In reality its caudal expansion becomes continuous with a mesodermal thickening—crescentic in form on surface-inspection—which is found at the posterior limit of the embryonic area.

(*r*) The anterior half, or thereabout, of the primitive streak lies within the lanceolate posterior prolongation of the paraxial region of the embryonic area. The remainder of the streak traverses the posterior segment of the parietal zone of the same area.

(*s*) The primitive streak is more or less grooved on its upper surface, but in an inconstant fashion.

(*t*) There is no indication whatever, on surface-view, of the differentiation of a medullary plate. Its absence is confirmed by the examination of the serial sections.

(7) "Neurular Stage,"* Characterised by Differentiation of Medullary Plate, etc. Specimens Z, ZZ, M and H.

The further developmental history of the embryonic area has been followed in our investigation as far as the stage which was described some years ago by Hill and Martin ('94). We are absolved by that description from the necessity of presenting a complete account of the stage referred to. But in several directions Hill and Martin's description requires amplification and correction.

An additional specimen (lettered "H" in our catalogue of material) has been obtained, which has proved in some respects more favourable for the purposes of investigation than the earlier described specimen. The serial sections of the latter have also been at our disposal, as well as the photomicrographs of the blastoderm which were taken prior to embedding. This embryo we shall refer to under the designation of "Specimen M."

The stage represented by these two embryos (*i.e.*, Specimens H and M) is characterised by the presence of certain well-marked features of neural organisation, viz., the complete differentiation of the medullary plate and of the primary ganglionic Anlagen; as well as by progressive differentiation of the mesoderm, including the distinct appearance, in the paraxial zone, of a long series of definitive somites.

We have also more recently obtained two twin specimens which exhibit the same general features of organisation as are manifested in the specimens above referred to, but in a decidedly earlier phase of development. All four specimens may be regarded as belonging to the same general stage, the two pairs of specimens representing earlier and later phases or sub-stages. And inasmuch as the characters of neural organisation constitute the most outstanding features possessed in common by these embryos, it is perhaps permissible to apply to the entire stage the designation of "neurular stage." The two phases represented by the two somewhat diverse pairs of embryos may conveniently be distinguished as those of the "early neurula" and "late neurula" respectively.

The details of the neural organisation of this "neurular" stage in Ornithorhynchus will receive full consideration in a subsequent section of this paper.

For information regarding certain other structural characters, reference must be made to the former account by Hill and Martin. For the present we propose to trace into this later stage the developmental history of some of the structures in and around the embryonic area which have already occupied our attention in connection with the anatomy of the postgastrular stage.

* We are by no means unconscious of the fact that a certain arbitrariness is involved in the selection of such terms as "gastrular," "postgastrular," and "neurular," in order to designate certain assumed "stages" in the developmental process. Our chief justification must be the great convenience resulting from the use of appellations which convey an idea of salient features of the temporary organisation of a continuously changing structural complex.

(a) *Description of Individual Specimens of Neurular Stage.*

The period of development now to be considered is characterised by the differentiation of evident somites, of the primary ganglionic rudiments, and of the medullary plate. The latter, throughout the whole of the period to which we refer, remains spread out flat, without any indication of the formation of a medullary groove. The entire embryonic and peri-embryonic differentiation remains as yet in the form of an elongated oval area of the spheroidal wall of a blastodermic vesicle.

(a) *Specimens Z and ZZ.*—The two earlier specimens in our possession belonging to this period were obtained from twin eggs and appear in our list of material under the distinguishing letters "Z" and "ZZ" respectively. The condition of both of these specimens unfortunately left much to be desired from the histological point of view. This we believe to be owing to some neglect on the part of the collector, which must have allowed of a partial slight desiccation of patches of the blastoderm. Portions of it are excellently preserved, even showing good mitotic figures; but elsewhere the cellular layers are unduly condensed and attenuated through shrinkage. This partially defective histological condition does not, however, substantially detract from the value of the specimens for examination, *in toto*, of their structural characters, and even, to a more limited extent, for sectional examination.

The eggs were received from the collector, after fixation, in a more or less collapsed and crumpled state. Specimen ZZ was, as a whole, in better condition. It was cautiously redistended in alcohol by gently injecting it through a hypodermic syringe. When thus treated, the egg had a maximum diameter of 14 millims. It was next opened up with scissors and the embryonic region of the wall of the blastodermic vesicle cut out. Fig. 73, Plate 1, shows a photomicrograph by reflected light of the ventral aspect of the latter, after the adoption of drastic measures to secure a moderate flattening of the area. Even then the irregular curvature of the surface was not wholly eliminated, as the out-of-focus patches prove. This figure should be compared with the photomicrograph of the Twin-specimen Z taken by transmitted light after clearing in cedar oil (fig. 74).

The total length of the embryo in Specimen ZZ, measured from the anterior border of the medullary plate to the posterior extremity of the primitive streak (which is not clearly visible in the photograph) was 14·5 millims. The length of the primitive streak itself was 2·2 millims. A "HENSEN'S knot" was distinctly recognisable. This measured 0·3 millim. in length. In continuity with it the chorda extends forwards, in the embryonic axis, into a gradually widening "protochordal-plate" expansion in the anterior cephalic region. This attains a maximum width of about 0·1 millim., and appears to fade away very shortly behind the median anterior limit of the medullary plate.

Twelve pairs of somites are fully differentiated, in Specimen ZZ, whilst in Z only 11 pairs are fully delimited. The paraxial segmented zone containing these somites

is seen to be continued on each side posteriorly into an ovate area traversed by the anterior portion of the primitive streak. This ovate area is limited, both laterally and behind, by a peripheral zone, which appears light and irregularly mottled in the photograph by reflected light in fig. 73. This is obviously continuous, anteriorly, on either side with the embryonic "parietal" unsegmented zone; whilst its peripheral limit coincides, as the serial sections show, with the inner limits of the area opaca of yolk entoderm.

In the posterior trunk-region the Wolffian duct may be recognised along the outer edge of the somites. It begins to be differentiated opposite the sixth somite. The serial sections show that a definitive chorda is established, or is in course of establishment, except quite anteriorly, where a flattened chorda-plate merges into the "protochordal-plate" expansion. Posteriorly, too, the definitive chorda is simply continued into the relatively wide and thick HENSEN'S knot.

In front of the medullary plate and extending backwards on either side at some distance from its lateral margins is a narrow and sharply-marked crescentic thickening (fig. 74). This coincides with the site of the commencing amniotic fold and represents the canal-like amnio-cardial space, which merges posteriorly in the pericardial region (*cf.* HILL and MARTIN, figs. 2–7, Plate 10).

(b) *Specimens M and H.*—The general characters of the two specimens M and H, representing the later phase of "neurular" organisation, may now be briefly considered.

Specimen M is the embryo formerly described and figured by HILL and MARTIN.* It is very slightly more advanced than Specimen H, although they both belong substantially to the same stage of development. We reproduce herewith, in fig. 75, Plate 1, a photomicrograph of Specimen M, in which the finer details are more adequately represented than in the figure formerly published.

This fig. 75, from Specimen M, should be compared with figs. 76 and 77, which are reproductions of photomicrographs of the anterior and posterior portions of the embryonic area of the other representative of the stage, Specimen H. The comparison is, however, subject to the qualification that fig. 75 represents a ventral, and figs. 76 and 77 the dorsal, aspect of the respective embryos. Since both were photographed as transparent objects, by transmitted light, this distinction is of minor importance. It must further, however, be noted that the magnification is different in the two cases, viz., 6 diameters in fig. 75 and 8 diameters in the case of figs. 76 and 77.

* In HILL and MARTIN'S account this embryo is described as being "from the egg just ready to be laid." We now know that this determination is probably incorrect, inasmuch as an Echidna embryo in our possession, removed from the newly laid egg of a female in captivity, corresponds roughly with a chick at about the end of the third day's incubation. CALDWELL, however ('84), has compared the embryo from the new-laid egg of an Ornithorhynchus to that of a chick of about thirty-six hours incubation.

Both eggs were intra-uterine eggs, measuring 18 millims. in their maximum diameter. Specimen M, and presumably also Specimen H, was found to possess 17 or 18 pairs of somites. The general features of the blastoderm in these two cases were very similar. That of Specimen H, however, had, unfortunately, suffered mutilation during transit, and a portion of the middle region of the embryo was irretrievably damaged.

Detailed consideration of the neural organisation of these embryos will be found in a later section. It will suffice in the meantime to point out that, in this stage, the medullary plate is well differentiated throughout, and exhibits clear neuromeric segmentation in the cephalic region. Posteriorly this plate widens into a leaf-like, or lanceolate expansion, which tapers away towards its hinder extremity (fig. 77). Everywhere the neural plate, or medullary plate, is flattened; nowhere, except in the anterior cephalic region, is there any indication of the uprising of medullary folds.

The primitive streak is well represented in the photomicrograph by transmitted light, reproduced in fig. 77, Plate 1. As compared with the corresponding structure in the postgastrular stage, it has undergone marked reduction in length, not only relative but absolute. Its longitudinal measurement is now only 1·58 millims. Of this length, about two-thirds is now included in the middle line of the hinder half of the lanceolate caudal expansion of the medullary plate. Its posterior third is represented by a rapidly widening triangular expansion. This is shown, by its lack of opacity, to possess a more attenuated character than the anterior two-thirds of the streak. The posterior, or basal extremity, of the expanded hinder third of the primitive streak meets the concavity of a crescentic opacity, due to the presence of a mesodermal thickening.

The crescentic opacity just mentioned is seen (fig. 77) to form the sharply accentuated hinder boundary of a tolerably broad zone, which is traceable anteriorly, on either side of the segmented paraxial zone, and which is therefore obviously the "parietal zone" of the embryo. In fig. 77 this zone is further recognisable through its somewhat deeper shade-differentiation from the region which borders on it peripherally.

All the features so far remarked upon are identifiable with features pointed out in connection with the surface-anatomy of the embryonic region of the type-specimen of the postgastrular stage, except that, at that earlier period, the differentiation of the medullary plate had not yet set in. There, in the earlier stage, as here in the later, the posterior portion of the primitive streak traversed the posterior continuation of the parietal zone. There, as here, it widened out at its hinder extremity (forming thus the so-called "caudal knot"). There, also, the expanded posterior end of the streak met the concavity of the crescentic mesodermal thickening. In the postgastrular stage, however, the anterior half of the primitive streak traversed the tapering posterior portion of the area of thickened ectoderm, which then covers, and

practically coincides with, the paraxial zone. It now traverses the lanceolate terminal segment of the medullary plate. It might be suggested that the tapering posterior portion of the area of thickened ectoderm, which we have termed "paraxial," is probably nothing but the "lanceolate terminal segment of the medullary plate" in process of differentiation. This, however, is not the case. The lateral limits of the thick "paraxial" ectoderm in the postgastrular stage lie considerably beyond the future lateral boundaries of the medullary plate. Posteriorly only, the hinder extremity of the medullary plate, when that does eventually develop, seems to occupy the whole of the posterior-pointed tapering segment of the "paraxial area" mapped out as such in text-fig. 8. The posterior coincidence of these regions is recognisable on careful examination of fig. 77.

(b) *Microscopical Anatomy of Neurular Stage, exclusive of the Neurological Characters.*

(a) *Embryonic Region Generally.*—Microscopical examination of transverse sections of this specimen shows that, at the outer limit of the parietal zone, the practically yolk-free entoderm of the embryonic or pellucid area gives place to the heavily yolk-laden entoderm of the area opaca. The region of the area opaca immediately surrounding the parietal embryonic zone shows, in the photograph in fig. 77, clearer and less opaque than the blastoderm surrounding it, or even than the embryonic parietal area which it encircles. But it is, nevertheless, genuine area opaca, so far as its entodermal character is concerned. Its greater transparency, in comparison with its surroundings, is entirely due to mesodermal thickening, both centrally and peripherally to it.

In the parietal zone the mesoderm is of no very great thickness. It is distinctly differentiated into two layers, somatic and splanchnic.

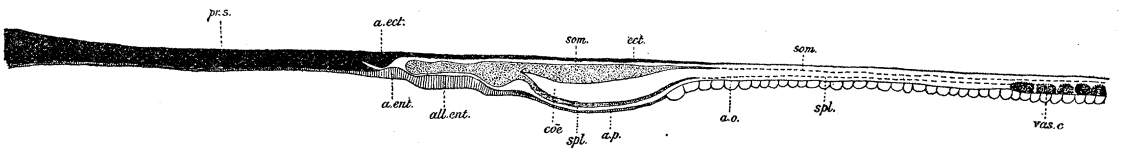
The two layers are, for the most part, in contact, without the intervention of any obvious cœlomic cavity. The splanchnic layer is very attenuated, consisting, almost throughout, of a single layer of flattened cells in contact with the entoderm. The somatic layer is from two to three times as thick, and possesses several strata of nuclei. (From the thickened somatopleure of this region are ultimately produced the limb-ridge and the definitive body-wall.) The posterior crescentic thickening, which forms the caudal margin of the parietal zone, is also seen to be due simply to a marked increase in the thickness of the somatic layer of the mesoderm, though the splanchnic layer is here also slightly thicker than in the more anterior portions of the parietal zone. Immediately behind the crescentic thickening both somatic and splanchnic layers, though still severally distinct, become reduced to single layers of attenuated cells. At the same time the underlying entoderm becomes yolk-laden, the area opaca having now been entered. It is thus evident that, as in Specimen P of the postgastrular stage, the crescentic thickening of mesoderm lies at the boundary between area opaca and area pellucida, but it is now evident that it

belongs properly to the latter. There is now also a superficially evident separation between the crescentic mesodermal thickening and the edge of the protangioblastic zone behind it and concentric with it. As the horns of the mesodermal crescent are continued forwards into the parietal zone, so the protangioblastic zone is continued forwards parallel with, and at some distance from, the latter. The interval between the parietal and protangioblastic zones constitutes that inner clearer zone of the area opaca which was described above. In the relatively opaquer "protangioblastic" zone are now appearing the actual Anlagen of the vascular system of the yolk-sac.

The posterior extremity of the primitive streak, after widening out in triangular form, meets the concavity of the crescentic mesodermal opacity, as has already been pointed out. The triangular expansion of the streak is somewhat thinned out, appearing transparent by transmitted light. Just at the posterior margin of this clear area is being formed the anal membrane, whilst the anterior portion of the crescentic mesodermal thickening itself, behind it, marks the site of the allantoic formation. The tail-fold of the amnion also arises, later, just over its anterior margin, between the anal membrane and the allantoic crescent. Directly underlying the anterior edge of the allantoic crescent is a limited area of thickened, but yolk-less, entoderm, whose appearance without doubt heralds the formation of the entodermal allantois. Immediately in front of this the entoderm of the anal membrane also exhibits a localised thickening.

In order to illustrate the sectional appearance of a number of the features described above, either from the surface-view or from study of the serial transverse sections, we have reconstructed from the latter a schematic representation of the median sectional plane in this region of the blastoderm. This is shown in text-fig. 14. The scheme includes the median section of the posterior, thinner, segment of the primitive streak, from the plane of the posterior extremity of the medullary plate backwards. In the opposite direction it reaches backwards to, and includes, the anterior margin of the vascular zone behind the embryonic area.

From this scheme it is evident that the parietal cœlomic cleavage of the mesoderm in the future allantoic region is incomplete (the depth of the cœlomic space is



TEXT-FIG. 14.—Schematic Mesial Plane-reconstruction from the Serial Transverse Sections of the Posterior Portion of the Embryonic Area in Specimen H.

pr. s., primitive streak, posterior end of; *som.*, somatic mesoderm; *spl.*, splanchnic mesoderm; *vas. c.*, vaso-formative cells; *a. p.*, entoderm of area pellucida; *a. o.*, entoderm of area opaca; *ect.*, ectoderm; *all. ent.*, allantoic entoderm; *a. ect.* and *a. ent.*, thickened ectoderm and entoderm of anal membrane. Magnif. = × 100.

purposely exaggerated throughout the scheme). The cœlomic cleft extends forwards, from the area opaca, for a certain distance under the thick somatic mesoderm of the allantoic crescent; but it stops short of the allantoic region proper, the mesoderm remaining unsplit where it overlies the allantoic thickening of the entoderm. Not until the cleavage has extended forwards to a point close behind the anal membrane can the tail-fold of the amnion arise.

(b) *Primitive Streak.*—When the posterior expanded segment of the primitive streak is traced forwards through the serial sections it is found to undergo thickening as it enters the tip of the terminal lanceolate expansion of the medullary plate. The commencemement of this thickening is represented at the extreme left edge of text-fig. 14. Traced further forwards from this point the streak is found to occupy the medial region of an elevated plateau, whose prominent lateral margins mark the edges of the medullary plate. Medially, where the latter is formed of streak tissue, the medullary plate is not differentiated from the deeper cell-material. The structure of the intramedullary segment of the streak calls for no special remark. Where it abuts upon the posterior boundary of the archenteric or primitive-knot tissue its relations are essentially identical with those described for the postgastrular stage. These relations in the present Specimen H have been already noted, and are illustrated in text-fig. 12, p. 75, which represents a median-plane-reconstruction of the posterior part of the archenteron, and anterior end of the primitive streak, in this specimen.

It is remarkable that no marked or important change in structural arrangement has occurred in, or around, the archenteric knot during the period of transition from the postgastrular stage to that of Specimen H. Even the original dimensions of the knot are maintained without substantial change. The absence of such change is the more noteworthy since, during the intervening period, growth-changes of a markedly unequal character have taken place in the embryonic axis. There has meanwhile occurred a shortening of the primitive streak from nearly 6 millims. to about 1·58 millims., and at the same time the representative ot the archenteron, in front of the streak, has undergone lengthening from about 5 millims. to about 17 millims. These correlative changes are definitely suggestive of the occurrence of backward transmigration of the archenteric "knot," *i.e.*, of the short, posterior, "intact," segment of the archenteron. This change in position may be supposed to be effected in some way or other at the expense of the primitive streak; and, on the other hand, to be accompanied by a progressive differentiation and elongation of the archenteric axial plate in front of the knot. But inasmuch as we find the same evidence of mutual independence of primitive streak and archenteron, in the shape of structural demarcation between them, as we showed in the postgastrular stage (*cf.* text-fig. 12, p. 75), we are unable to accept the view that the primitive streak has been progressively *converted into* archenteric tissue. It must be remembered that the backward prolongation of the archenteron, which we assume to occur in the

transition from the gastrular to the postgastrular stage, must of necessity be effected without any such co-operation on the part of the material of which the anterior part of the primitive streak is composed. It would therefore seem superfluous to invoke such a co-operation in order to explain what is merely the continuance of a process begun at that early period. We would rather suggest that the maintenance, through a lengthy period of development, of the mass, and of the morphological character, of the archenteric knot is really the maintenance of a mass of proliferative material, organised on a definite and persistent plan, for the purpose of generating the growing archenteric (and chordagenetic) axis. The shortening of the primitive streak which has latterly occurred *pari passu* with the elongation of the archenteric axis is probably to be regarded as atrophic in character. Originating as a mesoderm-producing organ, the primitive streak has outlived this aspect of its function; its activity in this direction is now practically negligible, and has well-nigh ceased. Its atrophy, under these circumstances, is easily intelligible, and is by no means confined to its anterior extremity, as ought to be the case if its disappearance were merely due to its progressive incorporation in the growing archenteric or chordagenetic axis.

(c) *Archenteron and its Derivatives: Chordagenesis.*—The sectional anatomy of the archenteric structures in Specimen H now demands more detailed consideration.

Fig. 35, Plate 8, shows a photomicrograph of a transverse section through the posterior portion of the archenteric (HENSEN'S) knot, a short distance behind the blastopore. Here the limitation and comparative isolation of the knot-tissue from its ectodermal surroundings is apparent. Fig. 100, Plate 17, illustrates a section passing through the blastoporic aperture itself, and fig. 101 another section 0·09 millim. in front of the latter. Here the originally transversely extended archenteric lumen is represented by two canals, forming so-called "duplex chorda-canals." This double character is not continued in any constant fashion throughout the serial sections. The lumen is more often single, sometimes double, and frequently obliterated altogether. The lumination of the archenteron is upon the whole, however, more complete and pronounced than in the earlier stage represented by Specimens P and E. This is evident on comparison of the text-figs. 10–12. We are inclined to attribute this fact to mere diminution in quantity of the cellular material of the archenteron in the later stage, so that compression of the lumen is to a large extent absent. Obliteration of the lumen, or reduction from an actual to a virtual lumen indicated only by a line, is a common accompaniment of the more massive cellular development of the walls of the intact archenteron in the earlier epoch. It is easy to understand how a cessation of active cellular proliferation at a later period might result in an opening up or reopening and widening of the narrower actual or potential lumen occurring in the antecedent period.

Comparison of fig. 101 with figs. 52 and 53, and 59–62, will suffice to carry conviction as to the essentially similar character of the underlying structural conditions.

When the archenteron is traced forwards from the "knot" region, in Specimen H, it gradually diminishes in size (fig. 102, Plate 17). It has now become impossible to recognise the plane—so evident in the preceding stages—at which the loss of the ventral archenteric wall has previously taken place. When a plane is reached which we can determine as that of the anterior limit of the "knot," by reason of the marked diminution in calibre, and by comparative measurements with the earlier stages of the knot, we no longer encounter the abrupt transition in character to a flattened and denuded archenteric plate. Nor does the lumen of the archenteron now open ventrally into the cavity of the yolk-sac, at least in this Specimen H, as was the case in the earlier stage. (In the case of the other and almost identical, "Specimen M," HILL and MARTIN have formerly described and figured ('94, fig. 21, Plate 12) a small ventral opening into the yolk-sac still persisting even at this advanced period.) In the present specimen H, the once ragged region of ventral archenteric disintegration —which is such a striking feature in fig. 49 and in text-figs. 10 and 11—has been completely repaired and smoothed over; so that we now pass, without any abrupt or irregular transition, from the undoubtedly intact and luminated archenteron behind (fig. 101), through a tolerably rapidly diminishing intermediate portion (fig. 102) into a slender, rod-like, definitive chorda (fig. 103).

There can be little doubt that fig. 102, which is taken from a plane 0·25 millim. in front of the blastopore, represents a section through that posterior portion of the archenteron which has never suffered denudation of its ventral wall. As little doubt can there be that fig. 103, from a plane about 1·5 millim. in front of the blastopore, represents a part of the definitive chorda derived from the archenteric plate. It is evident that the stage of flattened and exposed archenteric plate—so clearly demonstrable in the postgastrular stage—has now quite passed away. Not only has a solid rod-like chorda been organised from the earlier flattened plate, but the continuity of the entoderm has now been completely restored across the median plane, shutting out the archenteric derivatives from any direct relationship with the cavity of the yolk-sac. Anteriorly the definitive chorda in Specimens H and M merges in the gradually widening triangular expansion of the so-called "protochordal plate." This is visible in figs. 75 and 76, Plate 1, in the transparent median area of the "Rückenfurche." The gradually widening archenteric expansion in front of the definitive chorda may be followed forwards, in the serial sections in Specimen M, as far as the hinder edge of the elevated medullary fold or rim which terminates the cephalic plate anteriorly. It attains a maximum width in Specimen M of 0·135 millim. It is this "protochordal plate" which is represented in HILL and MARTIN'S figs. 2, Plate 10, and 14, Plate 11, under the letters "*nch.*" A comparison of these figures with their fig. 16, illustrating a paramesial section, will show that the epithelium of the archenteric "protochordal plate" extends as far as the anterior limit of the pharynx, into the constitution of whose dorsal wall it would appear to enter.

It must further be borne in mind that the definitive chorda is not the sole product

of the archenteric plate of the previous stage. This was our justification for refusing to that structure the designation of "chorda-plate." It is only the medial strip of the archenteric plate which properly constitutes a "chorda-plate." The differentiation between medial and lateral strips of the archenteric plate has previously been emphasised (*cf. e.g.*, figs. 56 and 57 and the series of figs. 63 *et seq.*). Only from the narrow medial strip of the archenteric plate is such a definitive chorda formed as is shown in fig. 103. The remaining lateral strips of the original archenteric plate are converted into gastral mesoderm, as formerly described.

The marked attenuation of the definitive chorda, illustrated in fig. 103, harmonises with the fact of its derivation from the narrow "chorda-plate" only, *i.e.*, the median strip of the original wide archenteric plate. The manner of transformation of "chorda-plate" into definitive chorda is illustrated in the series of figs. 104–107, Plate 3.

These figures are drawn from serial sections of the anterior region of Specimen ZZ, which was slightly less advanced than Specimen H, but in which the process of neural differentiation had begun (*cf.* figs. 73 and 74, Plate 3).

In fig. 104 the chorda-plate is shown as a flat cell-plate of no great width. The section lay about 0·4 millim. behind the posterior extremity of the "protochordal-plate" segment in the cephalic region. Its ventral surface is still devoid of entoderm. From this plane the chorda-plate continues backwards, showing only trifling change for some little distance. Figs. 105–107 represent three consecutive sections taken at a distance of 1·25 millims. behind the plane of fig. 104. In these sections the attempt of the chorda-plate to curl itself so as to constitute the wall of a groove, with the ultimate aim, as it were, of producing a tubular structure, is noticeable. The attempt amounts, however, to no more than a futile indication of an underlying tendency. In fig. 105 the entoderm does not yet meet ventrally beneath the chorda-plate. In fig. 106 its continuity is established across the median plane. The transition to fig. 107 shows how the organisation of a slender rod-like chorda is being effected by mere rearrangement of the constitutive cellular elements of the Anlage.

(d) *The Somites.*—Fig. 108, Plate 17, reproduces a photomicrograph of a transverse section across the axial region of the embryonic area in Specimen H. The section passes through the somite which is seventh from the posterior end of the series. The characteristically flattened and laterally expanded form of the somite is well shown. Owing to their slight obliquity, there is slight overlapping of successive somites. In the section figured the extreme mesio-anterior angle of one somite is seen to constitute the more mesial portion of the paraxial mesoderm, whilst the major lateral portion of the latter is formed by the body of the preceding somite. As the figure shows, a cleft-like cœlom is present in the somite. Laterally to the latter is seen the cross-section of the intermediate cell-mass or nephrotome. Beyond this are the lateral plates of parietal mesoderm, the somatic lamina of which is the thicker, consisting of several layers of cells.

Chapter II.—Discussion of the Morphological Significance of some of the Special Features noted in the Foregoing Description.

The descriptive account of the characters of the egg of Ornithorhynchus during the stages that we have thus far fully investigated has now been concluded. In the following section we propose to discuss the morphological bearings of some of the special features which have been noted in the course of that description.

The discussion will be found to turn chiefly around the phenomena of the gastrular and postgastrular stages and their relations to one another.

(a) *The Resemblance of the Primitive Knot of Ornithorhynchus to that of Reptiles.*

In our earlier communication the attention of the reader was drawn to the striking resemblance between the primitive or archenteric knot in the Monotreme egg, and that found in several reptilian forms. Reference was made to figs. 9 and 13 on Plate 8 of Mitsukuri's ('93) paper, illustrating the Chelonian archenteron, as well as to papers by Davenport ('96), and Dendy ('99). To these references may be added the monograph of Ballowitz ('01).

A comparison of the figures cited (*cf.* especially Mitsukuri's fig. 9), with the photomicrographs reproduced in our figs. 9 and 10, Plate 4, can leave but little doubt of the essential similarity of the conditions depicted (*cf.* also Ballowitz' text-fig 13). Even the special character of the loose and somewhat indefinitely organised reticular tissue which constitutes the bulk of the knot, as seen in our figs. 9 and 10, is closely paralleled by the appearances in the reptilian figures referred to. Again, in Mitsukuri's figs. 13–15 (*loc. cit.*) there will be recognised, in the position of the posterior lip of the blastopore, a somewhat more solid mass of tissue which doubtless answers to the cellular mass occupying a corresponding position in our figs. 9 and 10. In our descriptive account of the archenteric knot in Specimen Y, it was pointed out that the probable representative of this cellular posterior lip was present in the form of a considerable plug of small-celled tissue, protruding slightly at the surface of what we regard as a widely open blastoporic depression (fig. 11, Plate 5; text-fig. 5, p. 52). The cellular mass in question is structurally continuous with the posterior part of the knot. In this connection reference may be made to figs. 5–7, Plate 3, of an earlier paper by Mitsukuri ('91). Of these, fig. 7, which represents a transverse section across the region of the posterior lip of the blastopore in Clemmys, may be compared with our fig. 11, representing the transverse section of the knot in our Specimen Y. If, now, in Mitsukuri's figure, the surface-depression were deeper and more abrupt, and if the denser tissue in the region of the depressed posterior lip were elevated into a fungoid mass filling the depression, the condition arrived at

would be closely similar to that manifested in Specimen Y, as illustrated in fig. 11, Plate 5, and in text-figs. 4 and 5 (pp. 51 and 52).*

(b) *Comparison with the "Primitivplatte" of* WILL.

In connection with our account of Specimen "alpha," we have described and figured (*cf.* fig. 5, Plate 2, and pp. 40 and 41), what we take to be the earliest phase of development of the "primitive knot" which has so far come under our notice.

The lenticular cake of cells, there shown, was stated to correspond, in our opinion, to an early "primitive plate" in WILL'S sense. But if that be so, the differentiation of entoderm underlying it is effected much more precociously than in any of the reptilian forms described by him. And, on the other hand, the differentiation of cubical ectoderm to form an "embryonic shield" in front of this "primitive plate" (*i.e.*, the rudiment of the primitive or archenteric knot), which is an exceedingly early, and apparently quite constant, phenomenon in reptilian forms, does not take place in the Monotreme type until after actual invagination of the primitive plate or knot to form the gastrulation—or archenteric cavity, as seen in figs. 9 and 10.

A careful perusal of WILL'S papers, more particularly of that dealing with Lacerta ('**95**), and of his illustrative figures (*e.g.*, figs. 28–33) will suffice to convince one of the essential independence of the two concurrent processes of differentiation of the "primitive plate" on the one hand, and of the "embryonic shield" on the other. The series of surface-figures (1–5, Taf. i) from the paper just cited, graphically illustrate the primitive independence of the two regions, as well as their gradual incorporation in a common embryonic area.

In the course of our description of the gastrular stage of the Monotreme egg, it has appeared that, although at the period dealt with there is an entire absence of ectodermal shield-differentiation in front of the primitive knot, there is nevertheless present, behind the knot, a tolerably extensive area, traversed by a lengthy primitive streak, and characterised by the cubical character of its ectoderm, as well as by the presence of a not inconsiderable mesodermal sheet. This "primitive-streak area" is, moreover, entirely unconnected with the primitive knot, being separated from it by a not inconsiderable extent of bilaminar blastoderm, whose ectodermal layer is still flattened and squamous like that of the non-embryonic vesicle-wall (*cf.* text-fig. 7).

A statement of the condition thus summarised formed the main thesis of our earlier preliminary communication. It was pointed out towards the close of that paper that the recognition of the facts then outlined would involve a reconsideration of the entire question of the significance of the mammalian primitive streak.

* BALLOWITZ ('**01**) has also figured an "Epithelpropf" very similar in histological character to that referred to in Specimen Y, though occurring in the stage corresponding to our "postgastrular" (*cf.* BALLOWITZ' text-fig. 39 and figs. 43–5, Taf. xxxiii). But the important difference is here noticeable that the "Epithelauswuchs" or "Epithelpropf" is formed at the anterior lip of the blastopore.

In the course of his monograph on Lacerta, already referred to, WILL has attempted to demonstrate hypothetically the mode of derivation of the primitive streak of mammals from the structural condition represented by the "primitive plate" of reptiles. To any such hypothesis we are compelled, on the ground of our observations on the Monotreme condition, to enter a *non possumus*. Inasmuch as the so-called primitive streak of reptiles would seem to be nothing more than a mere backward prolongation of the posterior end of the archenteric knot or "primitive plate," it is impossible that it should be strictly homologous with the primitive streak of Ornithorhynchus, which we have shown to arise as a separate and autogenous formation.

The complete homology of the primitive streak of the Monotremes to that of other mammals cannot be seriously questioned. Its resemblance to the latter in structural organisation is unmistakable, as our sectional illustrations sufficiently show. And whenever we reach that stage in Monotreme development which we have designated in this paper the "postgastrular," the essential identity with the ordinary mammalian condition becomes perfectly obvious. At this period one can have no hesitation in identifying the archenteric "knot," as schematically shown in text-figs. 8, 10, and 11, with "HENSEN'S knot" of other mammals, on the one hand, and, on the other, with the isolated and independent "primitive knot" of the preceding gastrular stage.

(c) *The Probable Mode of Derivation of the "Postgastrular" from the "Gastrular" Condition.*

The question now arises as to the nature of the process by which the "postgastrular" condition is derived from that preceding it. In a previous section of this paper it has been shown that this transition is characterised by (*a*) a relatively enormous prolongation of the archenteron, the primitive or archenteric knot being continued onwards by means of the archenteric plate; (*b*) by an extension forwards, from the "primitive-streak area," of the differentiative process which leads to the establishment of the "embryonic" or pellucid area as shown in text-fig. 8. This includes, as its posterior component, the original "primitive-streak area," whilst anteriorly it comprises the entire region traversed by the, now greatly elongated, archenteric axis. A further characteristic of the attainment of the postgastrular condition is (*c*) the intimate apposition of the anterior end of the primitive streak with the posterior boundary of the archenteric knot, so that an appearance of confluence or continuity is presented, as in the case of the ordinary mammalian primitive streak and HENSEN'S knot. Were it not for the fact of the original distinctness of these two structures in the preceding stage, the faint demarcation between them which persists at the present stage might possibly have been overlooked, in series of transverse sections. The serial photomicrographs, figs. 29–33,

Plate 7, represent the critical sections through the hinder end of the knot, of which text-fig. 10, p. 73, shows a mesial-plane-reconstruction. These yield evidence sufficient to prove that the knot retains its individuality even at a period when, on surface view, it may appear to have become incorporated with the anterior portion of the primitive streak.

When we take account of the relationship subsisting between the primitive streak and the archenteric or primitive knot in the gastrular and postgastrular stages, respectively, we are compelled to assume that, during an intervening period, there must occur either a considerable forward extension of the anterior end of the primitive streak, or a backward transportation of the primitive knot, or a combination of these processes, whereby the two structures, independent in origin and growth, are finally brought into direct topographical relationship with one another.

Unfortunately the material at our disposal has not included a single specimen belonging to this intermediate period. We have had at our disposal four specimens from the gastrular stage, with isolated primitive knot and an absence of forward archenteric extension. And we have also obtained an equal number of "post-gastrular" specimens, each of which possesses an elongated archenteric plate, measuring several millimetres in length, which must have arisen by elongation of the archenteric knot of the gastrular stage. But we have no example of a growing archenteron of intermediate length, or of one in which the primitive-streak differentiation has approximated very closely to, but has not yet come into apposition with, the hinder boundary of the archenteric knot. In these circumstances any judgment we may form as to the nature of the intervening growth-processes must be arrived at through inference rather than direct observation. It is therefore evident that comparative measurements are desirable, showing the lengths of primitive streak, archenteric knot and archenteric plate, in the postgastrular stage, as compared with the lengths of the primitive streak, primitive or archenteric knot, and intervening distance between these, in the gastrular stage.

Several of our specimens were from various reasons unfitted to yield fully reliable data on all of these points. But in the cases of the type-specimens P and Q we are able to give figures adequate for the purpose in view.

It has been stated that the total length of the axial structures in specimen P was 11·3 millims., to which the primitive streak contributed 5·8 millims. In the Specimen Q the length of the primitive streak was 6 millims. Between the anterior end of this and the anterior limit of the thick ectoderm of the "primitive-streak area," was a distance of 1·5 millims., and from the latter limit to the primitive knot a further distance of, approximately, 2–2·5 millims., giving a total extent of, approximately, 10 millims. Consideration of these measurements does not tend to support the hypothesis of the occurrence of any substantial forward growth of the head end of the primitive streak, in the interval between the two periods examined.

On the other hand, it would seem to suggest that the bridging-over of the original interval between primitive knot and primitive streak must in some way be due to a backward dislocation, or migration, of the blastoporic aperture, and of the archenteric structure in its immediate vicinity. Such a backward transmigration may be conceived as being consequent upon, and as proceeding *parri passu* with, the differentiation, in front of it, of the gradually elongating archenteric axial plate.

According to this view the anterior limit of the primitive knot of the gastrular stage should correspond approximately, in its position in the blastoderm, with the anterior limit of the archenteric plate in the postgastrular stage.

In any case we are bound to admit that the "primitive" or "archenteric" knot of the earlier stage is morphologically equivalent to much more than that posterior intact portion of the archenteron in the later (postgastrular) stage, to which we have quite frequently applied the like designation of "primitive knot." The latter is identical with "HENSEN's knot," and not this only, but the whole of the lengthy "archenteric plate" (head-process) as well, are to be regarded as the derivatives of the original "primitive knot" of the gastrular stage.

The facts set forth in the paper seem to indicate that the differentiation of the archenteric plate proceeds from before backwards, and that therefore the oldest part of the plate is its anterior extremity.*

It is true that, in the instances above cited, the distance in Specimen Q, measured from the fore end of the knot to the hinder end of the primitive streak, falls short, by approximately 2 millims., of the full extent of the axial differentiation in the subsequent postgastrular stage as represented by Specimen P. But it must be borne in mind that, during the transition from the earlier to the later stage, the dimensions of the blastodermic vesicle have increased from about 9 or 10 to about 12·5 millims. in diameter. It is therefore reasonable to suppose that interstitial growth would in large degree affect the longitudinal extent of the area in question.

(d) *Comparison of the "Embryonic Shield" in Reptiles with that of Mammals.*

We have repeatedly referred to the absence, in the early stages of Monotreme development, of any such ectodermal "shield" differentiation, in front of the primitive knot, as is to be found in reptiles. On the other hand, we have seen

* In this connection it has also been pointed out that in the neurular Specimen H the structure of the blastoporic region and intact portion of the archenteron are practically identical with those of the post-gastrular stage. Yet in Specimen H there has meanwhile occurred an extensive differentiation of definitive notochord throughout no less than 17 millims., associated with a reduction in length of the primitive streak behind the knot to about 1·5 millims. This fact strengthens the necessity for the assumption of a further backward migration of the posterior end of the archenteron, this time at the direct expense of the primitive streak. And, if this be so, the presumption will be strengthened, that the greater part of the elongation of the archenteron which has taken place since the period when the primitive knot was situated well in front of the primitive-streak area, may have been effected by the operation of a like process.

that an area of apparently analogous character does arise, at an early period, altogether behind the primitive knot; and that this is traversed by the autogenous primitive streak. It is obvious that this "primitive-streak area" cannot be regarded as truly homologous with the "embryonic shield" of the reptilian ovum.

On the other hand, the so-called "embryonic shield" of the early mammalian blastodermic vesicle is pre-eminently an area of ectodermal differentiation, which is, throughout a large part of its extent, traversed by a relatively lengthy primitive streak. The appearance of the latter is commonly prior to the appearance of a primitive or HENSEN's knot. And in many cases, indeed, the anterior end of the streak at the period of its maximum extension is not far removed from the anterior limit of the "shield" (*cf.* KEIBEL ('93)).

This characteristic mammalian condition cannot without great difficulty be derived from the reptilian type of "embryonic shield," and the consideration of the case of Ornithorhynchus supplies an additional argument against forcing such a homology. The so-called "primitive streak" of reptiles, constituted as it is by the posterior extremity of the primitive or archenteric knot, is in our opinion not really homologous with the linear area of mesodermal proliferation which forms the "primitive streak" in the gastrular stage of Ornithorhynchus.

The belated appearance of a "HENSEN's knot" at the anterior end of the primitive streak, in many mammals, is a phenomenon which is equally difficult of explanation, whether we have regard to the reptilian or to the Monotreme condition. But when it does appear in its typically reduced and "abbreviated" mammalian condition, it is found to occupy precisely the same position to which it has attained in the "postgastrular" stage in Ornithorhynchus, through the processes of development which have been described above.

In the literature of avian and mammalian embryology it is usual to find the primitive or HENSEN's knot regarded as the thickened anterior extremity of the primitive streak, and there can be no doubt that in many cases it has the appearance of so arising. Nevertheless, both as regards birds and mammals, evidence has been adduced in support of its independent, and even of its primary, origin (*cf.* BONNET ('84)), MITROPHANOW ('02)). Even in Ornithorhynchus, where the mutual independence of primitive knot and primitive streak is so startling, these structures very rapidly come into such intimate relation that they soon appear as differentiated portions of a single axial structure. The knot is then found to be placed approximately in the middle of the, then greatly elongated, embryonic area. In view of this fact it appears to us that the apparent unity and continuity of the elements in question in other instances must be regarded with some reserve.

It must be admitted that in certain cases, both in birds and in mammals, there appears to be complete continuity, both materially and constitutionally, between HENSEN's knot, with its blastoporic aperture or depression on the one hand, and the primitive streak with its primitive groove on the other. Nevertheless, we here

insist that the facts of Monotreme development not only permit, but must be held to demand, an analysis of the knot-streak complex into distinct morphological elements.

(e) *The "Archenteric" Nature of the "Head-Process."*

A further corollary of the conclusions arrived at in this paper is that the time-honoured term "head-process" ought to be discarded as an inappropriate designation for the anterior prolongation of the archenteron (*cf.* also HUBRECHT ('02, p. 23, footnote)). As originally employed by v. KÖLLIKER, the term "head-process of the primitive streak" implied a view of its nature and origin which is inconsistent with the facts of Monotreme development. The distinctively archenteric character of the so-called head-process in reptiles has been abundantly illustrated in the records of recent investigation in that class. And that a similar interpretation of the mammalian "head-process" is the correct one is shown—altogether apart from the case of Ornithorhynchus—by, *e.g.*, VAN BENEDEN'S figs. 1–4 ('99), illustrating the morphological character of the structure in Vespertilio.

There is a general objection to the retention of the term "head-process," altogether apart from that founded on its original significance as a supposed primitive-streak structure. The additional objection to its employment is its ambiguity. The morphological value of a visible axial differentiation, in front of the site of the blastopore or its representative, may be different at different times, and in different regions. Thus it may signify (*a*) the forward prolongation, through a greater or less extent, of the intact archenteron possessing either an actual or a virtual lumen; (*b*) the dorsal remnant of the parietes of the cranially prolonged archenteron, after disintegration and disappearance of its ventral wall, constituting what we have referred to as the "archenteric plate" (BONNET'S "Urdarmplatte," '01); (*c*) the chorda-plate in the strict sense (BONNET'S "Chordaplatte"), *i.e.*, the axial strip or narrower cell-plate which remains after the differentiation of gastral mesoderm from the edges of the archenteric plate, but prior to the transformation into definitive chorda.

In the case of Ornithorhynchus, it has already been shown that the extensive axial differentiation in front of the blastopore in the postgastrular stage is constituted, in its most posterior portion, and for a very short distance only, by a persistent intact segment of the original archenteron. In front of this the "head-process" is a greatly elongated archenteric plate, ending anteriorly in a "protochordal" expansion.

(f) *The Mode of Genesis of the Archenteric Plate from the Primitive Archenteron.*

Our view of the genesis of the archenteric plate from the dorsal wall of a primitive archenteric cavity has been expressed in detail in the course of the preceding pages. A convincing demonstration of the actual occurrence of such a process is obtainable

from the sections illustrated in our figs. 44–49, 50, Plates 9 and 10. It is true that the region actually illustrated extends for only a very short distance in front of the region in which the ventral wall of the archenteron is still intact.

But we cannot entertain a doubt but that the entire archenteric plate in front has a similar origin, and that a disappearance of the ventral archenteric wall has taken place along the whole length of what was originally an elongated archenteric diverticulum, actual or potential. It is not necessary to hold that at any given period an actual lumen existed along the entire length of the long archenteron. The disintegration of the ventral wall may very well have proceeded *pari passu* with the progressive elongation of the archenteron.

The process of breaking through of the floor of an archenteric cavity, and of the production of an archenteric plate from its dorsal wall, is fully illustrated by BALLOWITZ ('01) in his monograph on gastrulation in Tropidonatrix (*cf.* especially his text-figs. 16 and 17).

The condition described and figured by LIEBERKÜHN ('84) in the guinea-pig offers a tolerably close parallel, in respect of the archenteric arrangements, to the "post-gastrular" condition in Ornithorhynchus. Figs. 25–30 on Taf. xx accompanying LIEBERKÜHN'S paper are especially worthy of comparison with our figures. They show practically the same organisation, although the term "Chordakanal" is applied to the archenteric lumen. We hold the latter term to be inappropriate because, in the Monotreme at least, and probably in all cases, it is only the medial portion of the dorsal wall of the canal thus designated which is destined to become converted into the definitive chorda. As shown by LIEBERKÜHN'S own figures, the ventral wall disappears, though it would seem that he interpreted its disappearance as due to an opening-up, and not necessarily to an actual disintegration of the ventral wall. His fig. 30 further shows that in the guinea-pig the entire extent of the archenteron is of the nature of a tubular diverticulum, as we also conceive it to be in Ornithorhynchus.

An entirely similar conception of the so-called "head-process" with its "Chordakanal," or archenteric cavity, is conveyed in VAN BENEDEN'S ('99) account of the corresponding stage of the embryo of Vespertilio. The figures of longitudinal sections illustrating this paper are thoroughly illuminating, and serve to connect the condition of the mammalian archenteron with that of the reptilian as exemplified, *e.g.*, in WILL'S figures of the archenteron in Platydactylus (*cf.* especially figs. 55–58, Taf. ix ('92)).

It is true that VAN BENEDEN'S figures do not show that posterior limitation of the archenteric tissue immediately behind the blastopore, which we have found in Ornithorhynchus (*cf.* our text-figs. 10–12), and upon which we have been compelled by the condition of the antecedent "gastrular" stage to lay such emphasis. We can easily understand that the existence of any such limitation at the stage figured may have been entirely unrecognisable.

In Tarsius, too, HUBRECHT ('02) has described and figured structural arrangements which are essentially identical with those above described. Especially do his figs. 72, 73, and 74, on Taf. ix, closely resemble in essential character the postgastrular stage, as we have called it, in Ornithorhynchus. And, in particular, the last of these figures (fig. 74) seems to show a definite trace of distinction and limitation between the anterior end of the primitive streak and the hinder part of the archenteric tissue lying immediately behind the blastoporic or archenteric (" neurenteric ") opening. Comparison may be invited between the figures here cited and our text-figs. 10–12, from Ornithorhynchus.

(g) *The Condition of the Monotreme Archenteron further illustrated from that found in a Marsupial* (*Perameles*).

We are enabled further to elucidate our conception of the constitution of the mammalian archenteron by reference to the structural condition found in a corresponding " postgastrular " stage of the blastodermic vesicle of a Marsupial (*Perameles obesula*). A photomicrograph of the upper surface of such a vesicle at the stage in question is reproduced in fig. 72, Plate 1.

The embryonic area was embedded and cut in series of vertical longitudinal (sagittal) sections. The entire archenteron is visible in the sections. It measured 3·2 millims. in length, from the posterior margin of the " knot-portion "(" primitive " or " HENSEN'S knot "), to the tip of the evident anterior extremity.

Figs. 68 and 69, Plate 13, show the caudal and cranial portions respectively of the archenteron. Of these, fig. 69 does not represent exactly the same section as that figured in fig. 68. Fig. 68 is from a neighbouring section, which, at the posterior extremity, was probably more accurately median in position. (It would indeed be impossible without a reconstruction, which has not, so far, been attempted, to decide which of several sections is most nearly median. The precise determination is in any case of little importance for our purposes.)

Towards the extreme left of fig. 68 is seen the anterior portion of the primitive streak. This is marked off by a slight notch, *n.*, from a tolerably lengthy cellular mass, *p. l. bl.*, which lies behind the very marked blastoporic depression, *bl. dep.* From the notch which thus marks the anterior boundary of the primitive streak, a line of demarcation may be traced in the section, downwards and forwards, between the primitive-streak tissue and that of the cellular mass in front of it which constitutes the thick posterior lip of the blastopore. Unfortunately, this region is not quite sharply focussed in the photomicrograph.

Fig. 70 represents another of the approximately median sections, and in this photograph the important distinction and delimitation of the primitive-streak tissue from the archenteric tissue in front is more clearly visible.

Immediately in front of the obvious blastoporic depression (*bl. dep.*), visible in both

of these sections, the knot undergoes some thickening (*cf.* also figs. 29–31, 37–39, and text-figs. 10–12). Here the undifferentiated archenteric tissue reaches the surface, forming the prominent bulging, whose posterior boundary forms the anterior lip of the blastopore. In front of this bulging there may be seen in both of the sections, figs. 68 and 70, a surface-depression, *s.d.* In front of this depression the differentiated ectoderm for the first time reaches the middle line in front of the undifferentiated tissue of the knot. In this connection the figures should be compared with the series of figs. 41–43. These illustrate exactly the same arrangement in Ornithorhynchus, only that, owing to the greater bulk of the indifferent tissue of the knot in the latter, the bulging is more prominent and there is no depression in front of it even when the ectoderm has become fully differentiated across the middle line. Here, in front of the prominence of the undifferentiated tissue of the knot, the ectoderm extends as a thick layer, beneath which the underlying tissue of the knot is prolonged forwards for a short distance as a thick "head-process," or, as we prefer to call it, an "intact archenteron." This latter shows, indeed, no actual archenteric lumen; but after a very short course its entire ventral moiety abruptly disappears, as is well seen in fig. 68, Plate 13. Prior to its disappearance, however, this ventral component or floor-portion of the archenteric parietes has become marked off from the dorsal component, or archenteric roof, by the appearance of a sharp and definite horizontal line of demarcation, constituting a virtual archenteric lumen. In this respect the condition here manifested is identical with that seen in text-fig. 11, from Ornithorhynchus. In front of the plane at which the archenteric "floor" disappears, the "roof" of the archenteron is continued forwards as an elongated axial cellular plate to the anterior extremity of the embryo. The anterior terminal segment of the plate is shown in the photomicrograph reproduced in fig. 69. [The sectional figures here referred to should be compared with the photomicrograph in fig. 72, which represents the surface-view, by transmitted light, of a Perameles blastoderm identical with the one represented in section in the figs. 68–71, to which, therefore, fig. 72 will to some extent serve as a key.]

(h) *The "Protochordal-plate" Segment of the Archenteron.*

Examination of the sagittal sections of the Perameles embryo reveals the fact that for a long distance forwards (viz., about 2·4 millims. measured from the plane of disappearance of the archenteric floor) the archenteric plate preserves a practically constant thickness. At a short distance (0·37 millim.) from its anterior extremity, however, the plate shows a very definite thickening; the sagittal section of this, its anterior terminal, segment showing a marked club-shaped form. The clubbed extremity is terminated cranially by a very distinct convex contour. This contour sharply limits it from the tissue of the entodermal sheet which replaces it in front. The thickened anterior segment of the archenteron, thus described, is readily distinguishable in

fig. 69. Here the commencement of the thickening is visible, not far from the left edge of the field; and from this point there is a gradual increase in thickness up to the actual club-shaped cranial extremity. This cranial extremity of the archenteron, club-shaped in sagittal section, is a very striking feature of the embryonic axis in this particular case. Its general shape and constitution, its massiveness, and its sharp distinction and limitation from the tissue in front of it, strongly support the view that it constitutes the fundus or extreme anterior end of the original archenteric diverticulum, which has here not entirely lost its ventral cell-elements. And this interpretation is well supported by a consideration of fig. 71, Plate 13. This figure represents a transverse section through the anterior knobbed extremity of the archenteron, in another Perameles embryo of the same stage as that represented in the preceding figures. Here the community of structural character between this anterior portion of the archenteron and the intact posterior portion of the archenteron in front of the blastopore, is sufficiently obvious.

Whether or not any more partial cellular denudation of the ventral archenteric surface has occurred in this most anterior region cannot well be determined. It may, however, be remarked that, on the hypothesis of a backward migration of the dorsal archenteric aperture, with concurrent progressive differentiation and elongation of the archenteron, the most anterior region of the latter would be, ontogenetically, the oldest; and hence the denudative process, if occurring here at all, might well enough be unrecognisable at the stage under consideration. But this thickened anterior segment of the archenteric parietes coincides with, and doubtless answers to, the so-called "protochordal plate" of Professor HUBRECHT ('02). Both in the sectional fig. 69, and in the surface-view in fig. 72, it is easy to map out this "protochordal-plate" segment. In fig. 72 it is recognisable both by its greater opacity and by its gradually increasing width. (Measuring, as has been stated, 0·37 millim. in length, its width increases from 0·05 millim., which is about the average width of the archenteric plate behind this anterior region, to 0·17 millim. near the thickened anterior termination of the plate.) But neither these figures, nor any observations we have made, lend support to the idea that this "protochordal-plate" area is different in origin or in essential character from the rest of the archenteric plate behind it. On the contrary, the appearances above described and figured are strongly suggestive of a preservation more or less intact of the extreme anterior segment, or fundus, of the primitive archenteron.* We venture to believe that an inspection of

* It is of no little interest to note that the maximum width attained by this "protochordal-plate" segment in Perameles, as illustrated in fig. 71, is just the same width as that exhibited by the "primitive-knot" segment posteriorly. According to the hypothesis adopted by us, this simply represents the original width of the archenteron. Where this width has undergone diminution, as is the case in Perameles throughout the long intermediate portion of the archenteric plate, or "head-process," this has been due to the conversion of the marginal strips of the original archenteric parietes into "gastral" mesoderm. Such a process we have already seen in actual progress in Specimen E of Ornithorhynchus (*cf.* figs. 65 and 66, Plate 12).

fig. 69 will suffice to carry conviction as to the essentially archenteric character of this "protochordal-plate" (?) segment of the axial differentiation. It is not, however, part of our present purpose to discuss at greater length the origin and significance of the so-called "protochordal-plate." On this subject reference may be made to works of HUBRECHT and BONNET (*loc. cit.*).

We have in a previous section (p. 62) drawn attention to the triangular or fan-shaped expansion in which the archenteric plate ends, cranially, in the postgastrular stage in Ornithorhynchus (*cf.* text-fig. 8). Unfortunately the region in question was missing from our Specimen E, and in neither of the other specimens are the sections completely satisfactory just at the transition from the archenteric-plate expansion to the entoderm into which that expansion passes anteriorly. Where the plate widens out we find that its constituent cells manifest a greater irregularity in form and arrangement than elsewhere in the plate. We have been unable to satisfy ourselves that this "protochordal-plate-like" expansion constitutes in any special sense a mesoderm-producing area. On the other hand, we see no reason to doubt that it may contribute to the production of "gastral" mesoderm, both from its lateral and its anterior periphery, after the same manner as we have seen this to occur along the margins of the archenteric plate behind this region. In any case we are disposed to interpret this "protochordal-plate" area in Ornithorhynchus on the lines suggested in connection with the interpretation of figs. 68–72, illustrative of the condition in Perameles.

(i) *The "Protosomites" in Ornithorhynchus, and* v. SPEE'S *former Observations on Similar Structures in Cavia.*

So far as our knowledge goes, the only investigator who has drawn attention to a differentiation of archenteric tissue similar to that disposition of bilaterally arranged, segmental, cellular, masses, which we have described above, as present in Ornithorhynchus, is GRAF V. SPEE ('88). Nor do we find in the embryological literature of the 16 years which have elapsed since the publication of SPEE'S paper, a single reference to the condition recorded by him as existent in the early guinea-pig embryo. This condition would appear to have closely resembled that observed in Ornithorhynchus by the present writers. The latter observations may be regarded as confirmatory of the former, both as regards SPEE'S clear and objective account of the structural features met with, and also as regards the general character of the interpretation suggested by that author. This is the more noteworthy in that we had arrived at, and formulated for ourselves, the same hypothesis, prior to our acquaintance with the contents of the paper by v. SPEE.

This interpretative hypothesis we have already expressed and embodied in our descriptive account by the introduction of the term "protosomite." By this term we have sought to designate, and thus more explicitly to recognise, a series of primitive

segmental masses, which appear as differentiations of the tissue of the walls of the posterior "intact" segment of the archenteron, *i.e.*, the segment usually known as the "primitive" or "HENSEN's knot."

It is unnecessary to recapitulate at any length the observations and views embodied in v. SPEE's paper. The following summary will suffice :—

In the entire region of HENSEN's knot (in the guinea-pig) the "neurenteric strand" is connected laterally with the outlying looser tissue of the mesodermal sheet. A cleft, or a contour line, is commonly met with, indicating a laminar differentiation of the tissue of the knot. In front of the knot the mesoderm is more sharply limited from the median chorda-producing tissue of the "head-process." (v. SPEE seems to have regarded this delimitation as a progressive one. For the present purpose the question is irrelevant.) Prior to delimitation of the chorda, peculiarities in the form of the "neurenteric strand" (*i.e.*, in our phaseology, the solid and intact archenteron), make their appearance (*cf*, v. SPEE's figs. 3 and 4). In v. SPEE's own words "Derselbe erscheint gleichsam gegliedert, abwechselnd eine Strecke weit mit einfacher, in der längeren Axe seines Querschnitts stehender, in der Mitte oder an den Enden erweiterter Spalte, oder aber in allen seinen Teilen ohne Massenzunahme verdoppelt." The latter condition is represented in v. SPEE's fig. 4, in which he shows, close beside one another, and symmetrically arranged with reference to the median plane, "zwei an fertige Urwirbel errinnernde, nur viel kleinere Gebilde." Each of these masses showed a central lumen surrounded by a cell-layer. Such double masses he found in one case within the knot in three places consecutively behind one another. Between these differentiated portions v. SPEE found structural arrangements transitional to the simple form of neurenteric strand and often difficult of interpretation. In his comments upon the condition, he remarks that it is difficult to conceive that these extraordinary structures should have no ulterior significance; and he proceeds to compare the condition with that of the doubling of the chorda-canal in later stages, suggesting a possible morphological similarity between the conditions involved. He then suggests that the repeated appearance and disappearance of the bilateral masses in question may possibly stand in closer relationship with the organisation of somites in the region of the knot. In this connection he relates his observation, in one case in the guinea-pig, of clear radial striæ extending obliquely outwards from the knot, and which perhaps indicated the beginnings of mesodermal segmentation. He also lays stress upon the obliquity of the early segmentation occurring in this region.

Since the date of v. SPEE's paper many notable advances have been made in the study of the phenomena of gastrulation and of the origin of the mesoderm, more particularly in reference to the Sauropsida. It is now possible to identify HENSEN's knot in the Mammalia, including the "neurenteric strand" developed from it, as well as the forward continuation known as the "head-process," with the well-developed, and often massive and laminated, archenteron of the Reptilia. We have seen in the present paper how striking is the resemblance between the archenteron of Ornitho-

rhynchus and corresponding stages of the reptilian gastrula. We have also shown that what we have termed the protosomites are in fact derivatives, *en masse*, from the archenteric knot; and that the cavities, actual or potential, found in their interior are always representatives, or remains, of that originally wide transverse archenteric lumen so well seen in our fig. 44, Plate 9.

Comparison of the foregoing account of v. SPEE's observations with our statements of the condition present in Ornithorhynchus can only lead to the conclusion that the phenomena in the two cases are essentially identical, and that v. SPEE was fully justified in his conviction that the appearances he met with were not due to merely fortuitous arrangements. The double masses of cells seen by v. SPEE are the same as those which we have designated protosomites in Ornithorhynchus. Of these we have observed in one specimen five more or less distinct and consecutive pairs, whilst v. SPEE saw three. The obliquity of their segmental arrangement is very pronounced in Ornithorhynchus and determines the appearance, in the same transverse section, of more than one pair at a time, since they overlap one another laterally. The transitional planes of section usually offer a confused appearance. Laterally the protosomites merge in the mesoderm, towards whose formation they substantially contribute. Mesially and anteriorly they are in series with the archenteric plate in front, and their mesial portions are very probably concerned in chorda formation, thus affording an explanation of the common occurrence of duplex chorda-canals. The archenteric region in which they are found seems to be exempt from the process of ventral denudation which lays bare the "archenteric plate" in front.

The term "protosomites" which we have employed in the designation of these masses is one which we are aware is liable to misinterpretation. We have definitely repudiated the idea (p. 88) that these masses are the first beginnings or Anlagen of the definitive somites. It has been pointed out that their area is not at all coincident with that broad paraxial zone of mesoderm which becomes the site of the, then impending, differentiation of definitive somites. On the contrary, the zone of origin of the protosomites is in fact coincident only with the lateral extent of the primitive archenteron, and they are represented further forwards by the lateral portions of the archenteric plate, in which, too, indications of a serial segmental differentiation are less definitely recognisable. The protosomites, or, at least, their major portions, do, however, ultimately merge in the neighbouring paraxial mesoderm, and this is likewise the case with their serial homologues, the marginal portions of the archenteric plate. But the mesoderm so contributed to the broad paraxial zone is trifling in amount, nor has this protosomitic segmentation any recognisable relation to the definitive somitic segmentation, numerical or other.

Our employment of the term "protosomite" really indicates our belief that the structures so designated are morphologically equivalent to palingenetic somites of direct archenteric origin, differentiated in and from the lateral walls of the

gastrula, and eventually incorporated in the general mesoderm. On the other hand, it is one of the chief results of the investigation recorded in this paper to establish the fact that in Ornithorhynchus the main mass of the general mesoderm, both parietal and paraxial, comes into existence wholly independently of the activity of the gastrular or archenteric walls. The incorporation in the somitic segments of the former, of the quite insignificant protosomitic derivatives of the latter, is therefore a union of structural elements which are *homoplastic* rather than *homogenetic*. And we are thus led up to the conception of a distinction between palingenetic and cenogenetic mesoderm which is precisely parallel to that often recognised between palingenetic and cenogenetic entoderm. It is, indeed, a distinction whose establishment has in all probability been conditioned in both cases by identical biological factors.

(k) *Summary.*

(*a*) There is a striking resemblance between the primitive knot of Ornithorhynchus and that of reptiles, and this resemblance would appear even to extend to certain points of detail.

(*b*) The primitive knot of Ornithorhynchus corresponds to the "Primitivplatte" of WILL, and the latter therefore does not find its homologue in the typically mammalian-like primitive streak of Ornithorhynchus.

(*c*) The postgastrular condition in Ornithorhynchus would appear to be derived from the antecedent gastrular one (1) through a remarkable prolongation of the archenteron; (2) by a rapid anterior extension of laminar differentiation originating more posteriorly in the "primitive-streak area"; (3) the establishment of an intimate apposition between the anterior end of the primitive streak and the posterior end of the archenteric (HENSEN'S) knot.

(*d*) The bringing about of the apposition just referred to is probably to be explained, at least in great part, by a backward transmigration of the knot-portion of the archenteron during the progressive elongation of the latter.

(*e*) The primitive knot of the gastrular stage represents much more than the HENSEN'S knot (also frequently termed "primitive knot") of the later postgastrular condition.

(*f*) The "embryonic shield" area of reptiles cannot be regarded as homologous either with the "primitive-streak area" of the monotreme gastrular stage or with the fully developed "embryonic area" of the postgastrular. Probably it corresponds with the anterior later differentiating sub-area of the latter.

(*g*) The so-called "primitive streak" of reptiles is not really homologous with the mammalian "primitive streak," as exemplified in Ornithorhynchus.

(*h*) The term "head-process" ought to be discarded, since the structure is not morphologically an anterior process of the primitive streak, as the term was originally intended to signify.

(*i*) The so-called "head-process" is merely the anterior prolongation of the archenteron, whose hinder intact portion is HENSEN'S knot.

(*k*) The archenteric plate originates as the dorsal wall of a primitive archenteric cavity, which is exposed after disappearance of the cellular elements of the ventral wall.

(*l*) The interpretation adopted of the monotreme conditions met with is further confirmed by comparison with longitudinal sections of the axial region of the embryonic area in the marsupial Perameles.

(*m*) The "protochordal-plate" segment of the archenteron is an integral part of the archenteric structure.

(*n*) It is possible that the denudation of its ventral wall may have been less complete than throughout the greater part of the archenteric plate.

(*o*) The bilateral series of cell-masses resulting from the rearrangement of the tissue of HENSEN'S knot (*i.e.*, the intact portion of the archenteron) is suggestive of a series of primitive segments of gastral mesoderm here designated "protosomites."

(*p*) These have nothing to do with the origination of the first definitive somites, nor are they in any way coextensive with the site of differentiation of the latter.

(*q*) They present an unmistakable resemblance to cell-masses described many years ago by v. SPEE in a corresponding situation in the guinea-pig.

CHAPTER III.—DESCRIPTIVE ACCOUNT, WITH DISCUSSION, OF THE EARLY NEURAL ORGANISATION OF THE EMBRYO IN MONOTREMES AND MARSUPIALS.

The stage of the Ornithorhynchus embryo which we have designated as the "neurular" has already been treated of, as regards its non-neural features. The neurological details of this highly-important stage will be found to repay a tolerably full and detailed consideration. Of the four specimens of the stage included in our collection, two manifest an earlier and two a later phase of the developmental phenomena which characterise the period. We have not thought it fit to distinguish these two phases as distinct and separate stages, since the outstanding features of the embryo are essentially the same in both the earlier and the later specimens.

(1) GENERAL NEUROLOGICAL DESCRIPTION OF EARLIER PHASE OF "NEURULAR" STAGE. (SPECIMENS Z AND ZZ.)

We shall now describe the earlier phase of neural organisation as manifested in the two twin-specimens Z and ZZ. These are illustrated in figs. 73 and 74, Plate 1.

(a) *Medullary Plate in General.*

In fig. 73, taken by reflected light, it is only the cephalic region of the medullary plate which is clearly visible. But in fig. 74, taken by transmitted light, the entire plate, with the exception of its mutilated caudal extremity, is easily recognisable. In this figure the cephalic region appears relatively wider than in the Specimen ZZ in fig. 73. But this is due merely to the irregular curvature of the imperfectly flattened blastoderm in the case of the latter. It is obvious in fig. 74, that, throughout the trunk-region of the embryo, the lateral portions of the medullary plate overlie the mesial halves of the paraxial segmented zones of embryonic mesoderm.

The cephalic region of the medullary plate widens in spathulate form as it is traced forwards from the region of the most anterior somites.

In both of the specimens the edges of the cephalic medullary plate are sharply defined, both in the anterior, and in the posterior, brain-regions. But, in the intermediate portion of the cephalic plate, the edges of the plate are practically indistinguishable. In the region in question the cephalic plate appears as if it underwent an abrupt widening on each side into a great lobate expansion. But this interpretation is not strictly correct.

(b) *Ganglionic Anlagen.*

The lateral lobes, which at first sight look as if they were alar expansions of the lateral edges of the medullary plate, are in reality covered over by thin ectoderm. This is continuous with the thick ectoderm of the medullary plate, along a line which is indeed not readily apparent in the photomicrographs, but which is the direct prolongation of the edge of the medullary plate in front of, and behind, the apparent interruption of that edge.

Nevertheless the serial sections show that the apparent continuity of the medullary plate with these lobate expansions at the sides of it is no mere fiction, but an expression of a genuine structural continuity between the deeper part of the edge of the thick medullary plate on the one hand, and the tissue of the subectodermal cellular plate, which constitutes the lobe in question, on the other hand. From its connections and relations there can be no doubt that this extensive, lobate, cellular, lamella is ganglionic in character; nor can there be any hesitation in identifying it with the primitive ganglion of the Trigeminus.

Further consideration of figs. 73 and 74 will show that what we have identified as the trigeminal ganglionic plate does not stand alone as a ganglionic rudiment in the head. Behind it, and opposite the anterior region of the hind-brain, there is to be seen, at the outer side of the lateral border of the medullary plate, a patch, dark by transmitted (fig. 74), and light by reflected (fig. 73), light. This we have ascertained

to be also a ganglionic plate, connected with the deeper part of the lateral edge of the medullary plate, from which it extends outwards under cover of the ectoderm of the head. It is, in fact, the acustico-facial ganglionic Anlage.

Caudally to the last-mentioned ganglion-rudiment, we also find, in both of the specimens (figs. 73 and 74), a narrow arcuate band, or strip, distinguished by its opacity. In fig. 74 this appears as if directly connected with the edge of the medullary plate, a short distance behind the acustico-facial ganglion; whilst, behind, it appears to merge itself in the paraxial zone, in the region of the most anterior somite. This appearance is, however, illusory. The strand in question is ganglionic and its connection with the lateral edge of the medullary plate is far more extensive and continuous than fig. 74 would lead one to suppose. In point of fact, the narrow opaque curved strand there visible, is only the somewhat thicker convex outer edge of an elongated ganglionic plate, which is the representative of the glossopharyngeus-vagus ganglion-complex. The more mesial portion of this elongated ganglionic plate is not wholly lacking, but consists of much looser and thinner cell-material, which therefore offers no increase of opacity when viewed by transmitted light.

When the ganglionic rudiments above enumerated are viewed as a series it will appear that they may be regarded as differentiations of, or outgrowths from, a "neural crest," which may presumably have been originally continuous, from the anterior limit of the Trigeminus ganglion-plate, backwards into the spinal region. Here, in the cephalic region, it is definitely discontinuous at two points. There is a marked hiatus between the Trigeminus and Acustico-facialis, and a lesser one between the latter and the Glossopharyngeus-vagus strand.

(c) *Neuromeric Segmentation.*

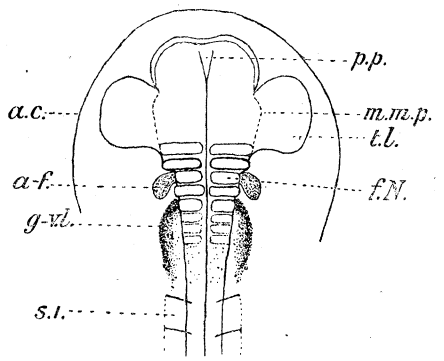
Yet another phase of neural development is also exemplified in the embryos under consideration. Reference to the illustrative figures will show (more especially fig. 73) that certain neuromeric segments have become differentiated, in the cephalic portion of the medullary plate.

One of these is specially noticeable in fig. 73, as a narrow whitish band crossing the medullary plate, on each side, just in front of the anterior limit of the acustico-facial ganglion. This neuromere is specially prominent not only in the present, but also in the succeeding stage. It may be designated provisionally as the "first prefacial" neuromere. Behind it there appears another, less distinctly indicated. With this the acustico-facial ganglion is seen to be connected. This quite definite connection enables us to apply to the neuromere in question the designation of "facial."*

* With almost equal justification it might be termed "auditory," since from it the ganglionic neuroblasts of the auditory organ appear to be proliferated. But the term "auditory neuromere" has by other writers (*e.g.*, ORR ('87) and MCCLURE ('95)) been applied to the neuromere lying immediately behind

This "facial" neuromere, although connected with the acustico-facial ganglion, is not that neuromere alongside of which the auditory plate is developed. The latter neuromere is also visible in fig. 73; and may be termed provisionally the "first post-facial" neuromere. The auditory plate itself can hardly be said to be visible in either of the figs. 73 or 74. It has, nevertheless, already come into existence as a patch of thickened ectoderm; and is located close to the edge of the medullary plate, immediately behind the connection of the acustico-facial ganglion with the latter.

The photomicrographs reproduced in figs. 73 and 74 do not reveal the full detail which was apparent under careful microscopical examination in suitable light, and especially with the stereoscopic binocular microscope. We therefore supply herewith a text-figure showing diagrammatically the outline of the details of organisation perceptible in the medullary plate in the region of the brain (text-fig. 15).



TEXT-FIG. 15.—Schematic Representation of Anterior End of Embryo in Early "Neurular" Stage, from Specimen ZZ.

a. c., line of amnio-cardial canal; *s.* 1, 1st somite; *m. m. p.*, lateral margin of medullary plate; *t. l.*, trigeminal ganglionic plate or lobe; *a. f.*, acustico-facial ganglionic plate; *g-v. l.*, vago-glossopharyngeal ganglionic plate; *f. N.*, "facial" neuromere; *p. p.*, "protochordal plate" expansion. Magnif. = × 6·4.

On careful microscopical examination it was possible to make out at least two prefacial neuromeres and four or five post-facial ones, although the most posterior ones were rather indistinct. But even behind the less distinct neuromeres and in front of the first somite there were present a series of very indefinite but apparently serially arranged opacities to which it was impossible to assign definite significance.

(d) *Indications of Definitive Organisation of the Medullary Plate.*

Indications of an approaching differentiation of the cephalic region of the medullary plate into regions corresponding to the future fore-, mid-, and hind-brain cannot with

this "facial" neuromere, in virtue of the topographical relationship of the former to the "auditory plate" of ectoderm; so that, in order to avoid confusion, it will be better entirely to avoid the use of the term "auditory neuromere."

certainty be held to be present. It is pretty certain, however, that by far the greater portion of the present cephalic plate belongs to the region of the future hind-brain. As regards its hinder limit we cannot accept, for higher vertebrates, the boundary which NEAL ('98) assigns to the "cephalic plate" in *Squalus acanthias*, viz., "the posterior boundary of the auditory invagination." A considerable extent of the medullary plate behind this must belong to the future myelencephalon; even if it should represent a region which is, phylogenetically, an annexe of the primitive cephalic plate.

Anteriorly, the limit of the future hind-brain is probably to be sought for at, or at least very close to, the anterior limit of the connection between the Trigeminus ganglionic lobe and the margin of the medullary plate. It is at this plane, too, that the chorda begins to widen into the expansion of the "protochordal plate."

(2) General Neurological Description of Later Phase of the "Neurular" Stage. (Specimens H and M.)

We may now turn to the consideration of the features of neural development which are displayed in the specimens representing the later phase of "neurular" organisation, viz., Specimens "H" and "M."

(a) *Medullary Plate in General.*

In this later phase of the neurular stage, as illustrated in fig. 76, Plate 1, the medullary plate in the entire cephalic region now shows well-defined lateral margins. Its anterior and antero-lateral margins further show a tolerably broad and prominent rim, which appears specially dark in the photograph. This is the optical expression of the commencing elevation of the margin of the forepart of the brain region above the plane of the blastoderm. The initiation of this process had, indeed, already become perceptible in the earlier period represented in fig. 74. In fig. 76 this rim seems as if it terminated posteriorly in a knobbed extremity, especially on the right side of the figure. But this is not actually the case, as examination of the photograph, especially with a magnifying glass, clearly shows. The "rim" really fades out just in front of the knob-like thickening. The opacity of the latter is found, on examination of the serial sections (*cf.* figs. 84 and 85), to be due to the proliferation of a mass of cells in this region, in connection with the edge of the medullary plate, and is not, like the rim in front of the knob, due to an uprising of the plate-margin. In the slightly more advanced Specimen M, the knob-like thickening has disappeared, as such, on both sides (*cf.* fig. 75).

(b) *Ganglionic Anlagen.*

The widely-expanded trigeminal ganglionic plates are here also, in this later stage as in the earlier one, very remarkable features of the head-region. But the

appearance of complete continuity with the medullary plate, which was so striking in the earlier Specimens Z and ZZ, has now been lost on surface-view, nor is the deeper connection with the edge of the plate now everywhere very definite and obvious in the sections. On the other hand, the trigeminal lobe is now seen to extend forwards almost as far as the plane of the anterior extremity of the cephalic medullary plate (*cf.* also fig. 78, illustrative of a like condition in Dasyurus).

In HILL and MARTIN'S account of the anatomy of Specimen M ('94), the real nature of the trigeminal ganglionic expansion was not appreciated; and the ganglionic plate was at that time interpreted by them as a "head-plate" of mesoderm. In their fig. 4, the cellular tissue lettered "hp. mes.," lying immediately underneath the ectoderm, really consists, predominantly if not exclusively, of ganglionic cell-elements; although, underlying this ganglionic tissue there are also present numerous scattered mesodermal cells. Comparisons of figs. 73 and 74 with figs. 75 and 76 will show that the continuity of the ganglionic plate with the medullary plate, which is so readily suggested by the earlier stage, is by no means so obvious in the later. A reinvestigation of the serial sections of Specimen M, along with the examination of those of the newer Specimen H, has further shown us that, in this later stage, the ganglionic cell-elements have become more dispersed and separated from one another; so that to ocular appearance the tissue is almost indistinguishable from mesodermal tissue. Further, in HILL and MARTIN'S Specimen M, the original cellular continuity, between the trigeminal ganglionic expansion of the neural crest and the edge of the medullary plate, has seemingly disappeared to a somewhat greater extent than even in the nearly related Specimen H; and this in turn shows a far less intimate and extensive continuity than is present in the earlier stage represented by Specimens Z and ZZ. Nevertheless, even in Specimen M, and still more in H, we find in the sections, in certain planes, a definite persistence of the original connection between the ganglionic plate and the edge of the medullary plate.

As a matter of fact, HILL and MARTIN were influenced in their interpretation by SELENKA'S view of the mesodermal character of the unquestionably similar head-plates which he observed in the cephalic region in Didelphys ('86–7). They quote his judgment with regard to these, that they "gehören offenbar zur Urwirbelplatte des Kopfes." There can be no doubt that SELENKA was entirely mistaken in this judgment. We have specially investigated this point in various specimens of Dasyurus embryos collected by one of us (H.) in connection with his work on the embryology of Marsupials. In this marsupial form we find the clearest possible confirmation of the ganglionic character of the so-called "head-plates." This is strongly suggested in the surface-view of the embryo in fig. 78, and is convincingly borne out by the study of the serial sections referred to later on in these pages. The Trigeminus-plate in Dasyurus is, indeed, far more obviously ganglionic than even in Ornithorhynchus.

In both of the figs. 75 and 76 the Trigeminus-plate is seen to end abruptly behind,

in a transverse border, posterior to which is a clear area quite free from neural crest elements, and at the same time possessing a very scanty mesoderm. This clear area is bounded posteriorly by the acustico-facial ganglion and its pedicle. The ganglion itself appears as an oval dark patch lying at a relatively greater distance from the edge of the medullary plate than in the earlier neurular stage already described (figs. 73 and 74). The pedicle which connects the obliquely-placed oval ganglion with the edge of the medullary plate consists of a proliferating tract of cells. Just behind the place of attachment of the pedicle, the medullary plate appears slightly, but distinctly, narrowed. Lying in a bay bounded medially by the edge of this constricted portion of the medullary plate, laterally by the hinder part of the acustico-facial ganglion, and anteriorly by the pedicle of this ganglion, there is visible, in both of the figs. 75 and 76, a more or less rounded area of only slight opacity. This is due to the presence here of a more or less circular patch of thickened surface-ectoderm, constituting the "auditory plate."

If reference be made to Plate 9 *bis*, illustrating HILL and MARTIN'S paper (*loc. cit.*), it will be seen that in their representation of the same embryo as that shown in our present fig. 75, the reference-line "*aud.*" leads to the acustico-facial ganglion, which would thus seem to be identified with the auditory plate. This, however, is clearly an oversight, for that which they correctly figure in section (in fig. 6, Plate 10) as "auditory plate," is obviously situated close to the edge of the medullary plate, and not so far out as the ganglion labelled "*aud.*" in Plate 9 *bis*. The plane of the section illustrated in their fig. 6, Plate 10, lies just behind—and the section accordingly misses—the posterior extremity of the acustico-facial ganglion.

In the earlier Specimens Z and ZZ we saw that the acustico-facial ganglion was situated tolerably close to the edge of the medullary plate, with the deeper part of whose lateral edge it is in obvious continuity. But, even in that stage, the sections prove that, behind the neck or pedicle of the ganglionic thickening, there exists a differentiated area of thickened ectoderm representing the Anlage of the auditory plate.

Behind the auditory region in fig. 76 there may be recognised the representative of the ganglionic plate which was indicated in the preceding phase as constituting the rudiment of the glossopharyngeus-vagus nerve-complex (*cf.* also text-fig. 15).

This ganglionic plate is thin and imperfect, and is tolerably well defined only anteriorly and laterally, these marginal portions constituting the thickest portions of the sub-ectodermal ganglionic cell-plate. The anterior margin is contiguous to the posterior edge of the auditory plate, and it is this anterior margin which on surface view is most plainly seen to be connected with the edge of the medullary plate. The almost equally definite lateral marginal portion of the plate is coincident with the medial limit of the "parietal zone" of embryonic mesoderm, and indeed with the inner limit of the cœlom, which already occupies the parietal zone in this its pericardial region (*cf.* fig. 96).

Posteriorly the ganglionic plate under consideration shows no definite limitation, and appears simply to merge in the region of the first somite. If regard were had only to surface-photographs such as those reproduced in figs. 75 and 76, it would naturally be supposed that that which we here describe as a ganglionic plate in reality merely represents the forward extension of the paraxial zone of mesoderm.

Fig. 75 is more especially liable to such an interpretation, inasmuch as the surface-view can hardly be said to yield any suggestion of the presence of such a sub-ectodermal ganglionic plate as that described. Here, on each side of the medullary plate, we find an oblong area bounded behind by the transverse cleavage-line defining the first distinct somite; laterally by a tolerably definite boundary which is coincident with the inner limit of the parietal cœlomic cavity; medially by the edge of the medullary plate; and anteriorly by a well-marked opaque band, parallel with the pedicle of the acustico-facial ganglion, and skirting the posterior part of the periphery of the circular auditory plate. The broad elongated area so defined is in tolerably obvious series with the great Trigeminus-plate in front; and also with the acustico-facial ganglion and its pedicle. That it is *not* to be interpreted as the forward prolongation of the paraxial zone of mesoderm is evidenced by the fact that the true anterior prolongation of the somitic series is itself visible, in both of the figures (75 and 76), in the shape of a pointed prolongation of somitic tissue, occupying only the medial posterior quarter of the oblong area in question, and tapering off here into a pointed anterior termination.

Such a negative, of course, in no way involves or supplies a demonstration of the ganglionic character of the cellular material of the area in question.

The existence of a ganglionic lamina in the sub-ectodermal plane of the elongated interval between the auditory region and that of the first somite, is rather difficult to realise, unless recourse be had to study of the sections. The conception of the existence of such a plate is slightly less difficult in the case of the right side of fig. 76. On both sides of the figure, but more especially on the left, the thin and imperfect nature of the lamina, except in its anterior and lateral marginal portions, is revealed in the relative lightness and transparency of the remainder of the area occupied by the plate. It is owing to this that the ganglionic rudiment seems on surface-view of this specimen to possess the form of an opaque strand running outwards and then backwards, behind the auditory region, to fade away in the region of the first somite. It has already been pointed out in connection with the early neurular phase that the arcuate band of opacity visible in the corresponding situation in fig. 74 is to be interpreted as the thickened and convex edge of a ganglionic plate or lamina. This thickened and convex margin is already less sharp and definite in fig. 76 than it was in fig. 74. But in fig. 75, from Specimen M, it is only the anterior margin of the plate which is clearly defined as such. The lateral margin, which here runs backwards at right angles with the anterior margin, is now practically coincident with, and on surface-view indistinguishable from, the

medial limit of the parietal embryonic cœlom. In consequence of this, the presence of a ganglionic cellular lamina in the sub-ectodermal plane is not at all suggested by the appearances seen on examination *in toto* by transmitted light, as in fig. 75. But it is nevertheless a fact that the entire oblong area under consideration is occupied by cellular tissue which is, at least in part, of ectodermal origin, and which represents in an imperfect fashion the non-marginal or pedicular portion of the ganglionic lamina representing the glossopharyngeus-vagus complex. This fact will become clear during the subsequent consideration of the sectional anatomy of the region. Comparison may even now be made with advantage between fig. 76 and the sectional fig. 96, Plate 16, which represents a transverse section across the hind-brain region of Specimen H, and shows the ganglionic plate representing the vago-glossopharyngeal complex.

(c) *Neuromeric Segmentation.*

Both of the Specimens H and M show a much more definite neuromeric segmentation than was perceptible in the immediately preceding phase. Those neuromeres, however, which were definitely present in the earlier specimens are still easily identifiable. Here, again, that neuromere which is obviously connected by a distinct strand or pedicle with the acustico-facial ganglion may be utilised for the provisional designation of the series of neural segments now distinguishable.

Three post-facial neuromeres are differentiated with tolerable distinctness on each side of the hind-brain in both specimens of the stage; although the posterior limitation of the third, or hindmost, of these, is not very apparent in the photomicrographs in figs. 75 and 76.

The first, or most anterior, of the two post-facial neuromeres lies opposite the middle of the auditory plaque. It is the most prominent of the three.

The second post-facial neuromere shows a less pronounced opacity than the first; but in both of the specimens it is distinguished by the peculiar localised opacity of its outer portion, next to the margin of the medullary plate. The structural explanation of this appearance will appear in connection with the examination of the serial sections. In both of the figures (75 and 76) the lateral margin of this neuromere appears as if it were quite definitely connected with the opaque strand, already explained above as constituting the anterior thickened margin of the ganglionic plate of the glossopharyngeus and vagus. In the sections, an actual cellular connection between the outer edge of the neuromere and the anterior margin of the ganglionic tissue would appear to be lacking; but there can be little doubt that such a connection was originally present, representing a primary connection between the margin of this segment of the cephalic plate and the plate-like ganglionic Anlage of the glossopharyngeal nerve.

The third post-facial neuromere is, in all probability, to be identified as that

cephalic neural segment, which is related to the most anterior vagus element ("Urvagus").

Behind the last-named segment, the medullary plate, opposite to the remainder of the ganglionic plate of the glossopharyngeus-vagus, exhibits on either side a somewhat uneven mottling, which is probably indicative of the persistence of traces of further neuromeric segmentation. In the earlier Specimens Z and ZZ it was possible to distinguish four, or perhaps even five, post-facial segments; and there were also present possible indications of even a greater number (*cf.* p. 120, and text-fig. 15). It would appear, then, that in the present stage the neuromeric segmentation of this posterior region is fading away.

In front of the "facial" neuromere we find again, in this "later neurular" stage, that there is one neuromere, the first prefacial, which is differentiated with great definiteness. Its lateral margin projects somewhat beyond the general contour of this region of the medullary plate; but otherwise there is no appearance suggestive of cell-proliferation from its lateral edge. It lies opposite the large and distinct hiatus which intervenes between the trigeminal and acustico-facial Anlagen.

Still further in front, and opposite the tolerably extended base of the Trigeminus ganglionic plate, there are to be distinguished three neuromeric segments. These are broader, and at the same time somewhat less distinct, than those behind them. In Specimen M (fig. 75) the middle one of these three segments, *i.e.*, the "third prefacial" neuromere of Specimen H, appears to be undergoing sub-division into two. Further, in each of the Specimens H and M (figs. 75 and 76), an additional pair of neuromeric segments is certainly, though somewhat vaguely, distinguishable, in front of that constriction of the medullary plate which nearly coincides with the cranial limit of the base of the Trigeminus ganglionic plate. In the case of Specimen H the last-mentioned neuromere is definitely connected with the prominent knob-like thickening of the edge of the medullary plate, seen especially on the right-hand side of the cephalic plate in fig. 76, and already referred to on p. 121. Through this thickening the neuromere in question is continuous with the anterior margin of the base of the Trigeminus lobe.

It will thus be seen that, in the stage under examination, there are at least five pairs, and in one of the specimens possibly six pairs, of prefacial neuromeres; and that, of these, all except one pair lie behind the cephalic constriction just alluded to. This transverse constriction of the cephalic plate is very obvious in figs. 75 and 76. It is barely, if at all, recognisable in figs. 73 and 74, from the earlier Specimens Z and ZZ. Nevertheless it was quite visible in these specimens themselves. In them it practically coincides in position with the angle of junction between the anterior margin of the base of the trigeminal lobe and the lateral margin of the medullary plate. The latter margin is faintly, but continuously, traceable backwards across the base of the trigeminal expansion. In Specimen H the lateral margin of the medullary plate now stands out in strong contrast with the base of the

trigeminal ganglionic plate, which it crosses. Hence the notch indenting the medullary margin near the anterior limit of the base of the trigeminal lobe is likewise far more evident. Although the notch is situated quite close to the anterior limit of the trigeminal pedicle it does not actually coincide with that limit; indeed, the anterior portion of the wide pedicle is seen in fig. 76, Plate 1, to be connected with the lateral medullary margin, in front of the notch in question. Through this connection in front of the notch, the most anterior portion of the trigeminal pedicle is connected with the deep edge of that segment of the medullary plate which corresponds to the most anterior, or fifth, prefacial neuromere. The connection is mediated by the knob-like thickening already referred to, in connection with fig. 76, as resulting from an abundant localised proliferation of cells from, or at, the lateral border of the most anterior neuromere. This specially thickened cellular connection must be taken to represent, at this period, the furthest anterior limit of trigeminal continuity with the deeper layers of the medullary margin. In figs. 75 and 76 the trigeminal lobe or ganglionic plate is seen to have extended forwards beneath the ectoderm of the side of the head, far in front of the plane of the anterior border of the original connection of the lobe with the edge of the medullary plate (*cf.* fig. 74). The original connection remains as a narrower "pedicle" of the now forwardly expanded lobe.

The medial marginal portion of the anterior extension of the trigeminal lobe is seen in fig. 76 to present a greater degree of opacity than the remainder. The opacity is traceable forwards from the region of the proliferative cellular thickening which forms the most anterior connection between trigeminal plate and medullary margin. The serial transverse sections show that the opacity is due to an extension forwards of a thickish cell-tract in continuity with the above cellular thickening. Although best seen in fig. 76, this differentiation is also visible in fig. 75 from Specimen M. It is to be observed that no connection between this thickened medial margin of the anterior expansion of the trigeminal lobe and the apparently closely contiguous lateral margin of the anterior segment of the medullary plate occurs anywhere in front of the connection with the fifth prefacial neuromere above described. The necessary absence of any such more anterior connection will be readily apparent on examination of fig. 86, Plate 14, showing a cross-section through the anterior region referred to.

(d) *Indications of Definitive Organisation of the Medullary Plate.*

In the course of our description of the earlier specimens of this stage (p. 120 *et seq.*) we gave expression to the opinion that the anterior limit of the hind-brain must be taken to extend so far forwards as to include nearly the entire basal connection between the trigeminal ganglionic lobe and the margin of the medullary plate. The notch on either side of the medullary plate constituting a transverse constriction of the cephalic plate in the region of the anterior portion of the trigeminal

pedicle corresponds, in our opinion, at least approximately, with the site of the future isthmus.* This belief is based not only on a consideration of present relationships, but also on a comparison of a later Echidna embryo showing an early stage of completion of the brain-vesicles—a stage which is not otherwise dealt with in this paper.

In a general way, therefore, though perhaps not with complete accuracy, we may take the transverse constriction of the cephalic plate, visible in figs. 75 and 76, as indicative of the future subdivision of the mid- and hind-brains.

In the stage at present under consideration, the medullary plate is still spread out as a completely flattened plate, with the sole exception of its anterior and antero-lateral margin, which is slightly elevated to form an upstanding "rim." No trace whatsoever of any cranial flexure is yet visible, and there are no indications of any of the encephalic plicæ which v. KUPFFER ('03) and others have recognised as foreshadowing the subdivision of the permanent encephalic segments in other vertebrates, unless the transverse constriction of the cephalic plate answer to one of these. The comparative clearness and simplicity of neuromeric segmentation, associated with the absence of foldings of any other description throughout the entire extent of the cephalic plate in this stage of the monotreme brain, constitutes a striking feature of brain-development in this form. It would appear to give the most explicit negative to all theories which attempt to explain neuromeric segmentation as due to the operation of extraneous and accidental factors. And in this connection it may be pointed out, that the cephalic mesoderm in Ornithorhynchus is at this stage quite negligible as a form-determining element. It is present only as an inconsiderable sheet of loosely arranged cells, in which it is difficult or impossible to find traces of any segmental arrangement. It would hardly be too much to say that the cephalic plate in Ornithorhynchus, at the stage of development with which we are now concerned, offers a complete *schema* of the constitution of a vertebrate brain in one particular phase of its ontogeny. Nor do we see any reason to suppose that any of the various features of this phase of organisation which we have described must be put aside as the result of any marked specialism, or of the operation of tendencies conveniently summed up under the name of cenogenesis. Apart altogether from the commonly assumed primitive character of the monotreme stock, we may again recall the fundamental similarity in type of early neural organisation exhibited by the Marsupialia, as witnessed by the studies of SELENKA in Didelphys. Here, also, the relatively long persistence of a flat condition of the cephalic plate, as of the embryo generally, reveals a picture of neural development only less clear than that of the Monotremes (*cf.* also our own fig. 78, Plate 1, from Dasyurus).

Inasmuch as the proliferative connection of the anterior border of the trigeminal pedicle and the fifth prefacial neuromere comes to lie in front of the transverse constriction of the cephalic plate, we are induced to regard the neuromere in

* *Cf.*, however, the later discussion of this question on p. 151.

question as in all probability belonging to the mid-brain region. The plane of its anterior border practically coincides with the anterior limit of the definitive chorda, so far as that is established in Specimens H and M. At this point the transverse serial sections show that the chorda begins to widen out into its "protochordal-plate" expansion. This widening does not become visible in the figs. 75 and 76 until a little in front of the plane mentioned. It is best seen in fig. 75. Both the anterior end of the chorda itself and its "protochordal" expansion are situated in a relatively transparent median strip of the anterior cephalic region. This strip represents the wide "Rückenfurche." A portion of this in the future fore-brain region must give rise to the infundibulum. The boundaries of the latter are not yet apparent.

A close examination of the photomicrograph reproduced in fig. 75 will further enable the observer to recognise the presence of the optic groove. This will be found on each side, in the anterior segment of the medullary cephalic plate, in the form of a slender dark crescent, concave medially, and lying just median to the opaque medullary rim which here bounds the cephalic plate laterally. The anterior horn of the crescent appears, indeed, to merge in the rim, whilst its posterior horn simply appears to fade away at, or immediately in front of, the anterior limit of the mid-brain neuromere aforesaid.

(3) Neurological Features of the Sectional Anatomy of the "Neurular" Stage.

The organisation of the cephalic region in the stage under consideration may now be further elucidated from the results of a study of the serial sections. This may, however, be prefaced by a brief description of the sectional appearances met with, in connection with the medullary plate, further back in the embryo.

(a) *Spinal Region.*

We select for examination a section transverse to the axis of Embryo H, and passing through the region of that somite which lies seventh from the posterior end of the series. The sectional plane is easily determinable in fig. 77, representing the posterior fragment of the embryonic area, etc., of Specimen H.

In fig. 79, Plate 13, there is reproduced a photomicrograph, at a magnification of 350 diameters, of part of one of the serial sections in the region specified. The figure includes one half of the medullary plate, and the parts immediately adjacent to it.

Here it will be seen that the lateral margin of the medullary plate projects for a short distance underneath the relatively thin and one-layered general ectoderm, and is here more or less continuous with an accumulation of cells, which fills up the angular interval between the general ectoderm and the edge of the plate itself, and

which also extends, ventrally to the edge of the plate, for a short distance both mesially and laterally. This cell-mass is plainly ganglionic.

Fig. 80 illustrates the corresponding cell-group on the opposite side of the next section but one. Here it is slightly more massive, but rather more sharply differentiated from the edge of the medullary plate.

The cell-group in question is visible throughout the entire series of sections in the spinal region, and represents the "neural ridge" or "crest" or "ganglionic strand" (*cf.* HILL and MARTIN, '94, p. 55).

The sections illustrated in figs. 79 and 80 pass across one of the segmental ganglionic thickenings of the neural crest. Between these thickenings the crest may be greatly attenuated, and its continuity may even, here and there in the present stage, be interrupted. A longitudinal commissural strand connecting the ganglionic enlargements is, however, for the most part verifiable.

(b) *Region of Second to Fifth "Prefacial" Neuromeres.*

In the cephalic region, the neural crest is represented by the ganglionic expansions previously described as representing the Trigeminus, Acustico-facialis, and Glossopharyngeus-vagus nerves. The appearance of these in figs. 75 and 76 has already been described in detail. The interruption of the continuity of the neural crest in the region of the head by a marked hiatus between the Trigeminus and the Acustico-facialis, and by another, less marked, hiatus between the latter and the Glossopharyngeus-vagus ganglionic plate, has also been commented upon. It remains to illustrate these features by sectional detail.

In fig. 81, Plate 14, is reproduced a photomicrograph, at a magnification of 78 diameters, illustrating a cross-section through one side of the head of specimen H. (Our series of transverse sections through the head of specimen H is from the left side, including, however, the median plane. The right side of the head was cut longitudinally.) The section passes through the anterior margin of the second prefacial neuromere. It shows the medullary plate continuous at its lateral margin with the ganglionic plate of the Trigeminus, *t. gl.* The cells composing the latter form a tolerably compact sheet for some distance out, but further out still the arrangement is a loose one. The difference in arrangement may be largely due to separation of the cells during section-technique. (The sections were cut prior to our adoption of the method of double-embedding as a routine procedure in all such cases.) In any case an attempt at the enumeration of the ganglionic cells present in equal extents of the cell-plate, showing the compact, and the loose, arrangement, does not tend to support the idea that there is any marked difference in the proportion of cells present in the different areas. The outer limit of the ganglionic plate may easily be recognised, just short of the marked crease in the layers towards the lateral part of the figure. It will be observed that, where the arrangement of the ganglionic cells is a loose

one, the resemblance of the ganglionic lamina to a mesodermal sheet is a very striking one.

Fig. 82 is a photomicrograph, at an enlargement of 350 diameters, of the extreme lateral portion of the ganglionic plate of the Trigeminus, as met with in Specimen ZZ, belonging to the earlier neurular stage (*cf.* fig. 73). In this case the sections were cut after double-embedding in celloidin-paraffin; and, partly at least, as a consequence of this, the cells are found arranged in a more compact form. In particular, the outer edge of the ganglionic plate is sharply defined. The relatively thin lamina of cells lying between entoderm and ectoderm, to the right of the edge of the ganglionic plate, is composed of genuine mesodermal tissue, as are also some few flattened cells underlying the free lateral margin of the ganglionic plate.

Fig. 83 represents, also under high power (× 350) magnification, a portion of another section in the trigeminal region of Specimen H. It shows the lateral portion of the medullary plate, together with its connection with the basal portion of the trigeminal ganglionic expansion. The proportional extent of the various structures shown in the figure must be estimated by comparison with fig. 81. The plane of the section lies in the region of the third prefacial neuromere. The relation of the margin of the medullary plate to the general ectoderm as well as to the ganglionic lamina is clearly shown and should be compared with that shown in the spinal region in figs. 79 and 80.

Figs. 84 and 85 represent low- and high-power views respectively of a section through the plane of connection between the most anterior portion of the wide base or pedicle of the trigeminal ganglionic lobe and the margin of the medullary plate. The section passes through the most anterior (fifth prefacial) neuromere, just in front of the transverse constriction of the cephalic plate seen in fig. 76.

In sections through this region the continuity of the basal portion of the ganglionic plate with the edge of the medullary plate is complete. Here, in the figure, the basal portion of the ganglionic expansion forms in section a thickened plano-convex cell-cake, which tapers laterally into the thinner outlying region of the ganglionic plate; but, medially, is directly confluent with the medullary plate, without undergoing any very marked diminution in thickness. At first sight, indeed, this thick basal portion of the ganglionic plate looks as if it were a marginal portion of the medullary plate itself. It is readily seen in fig. 85 that this is not the case, but that the cell-mass in question is really covered over, superficially, by the general ectoderm, which is traceable over it in a medial direction as far as the true margin of the medullary plate, with which it becomes confluent.

It is the presence of the basal ganglionic thickening seen in section in figs. 84 and 85 that gives rise to the localised opacity already noticed in connection with fig. 76 as lying just anterior to the notch in the lateral margin of the cephalic plate. The section figured is, however, from the left side of the head, whereas the opacity is most marked on the right side of the specimen, as shown in fig. 76.

It may further be observed that the layer of general ectoderm of the head which in fig. 85 may be seen to overlie this region of special thickening of the trigeminal plate, is distinctly thicker than it is elsewhere over the base of the trigeminal plate. We are unable to give an explanation of this difference, although it may be pointed out that this thickened patch of surface-ectoderm is in series with a still more thickened area, further back, which constitutes the auditory plate. The differentiation here noted has practically disappeared in the slightly older Specimen M.

It was previously pointed out, in connection with fig. 76, that from the localised thickening, whose sectional significance has just been indicated, there is traceable forwards an elongated area of diffuser opacity, which is obviously dependent upon a thickening of the medial portion of the forwardly extending trigeminal lobe. This area, as seen in surface view in fig. 76, appears as contiguous to, or even as if confluent with, the rudimentary lateral medullary fold or "rim" of the cephalic plate. The actual relations are revealed in the sectional illustration in fig. 86. Here are recognisable the elevation and partial circumscription of the lateral margin of the cephalic plate. These are much accentuated by the presence in the section of the posterior extremity of the optic groove. This sharply limits and defines the medullary rim on its medial side. Laterally, at *m. m. p.*, is the true summit of the medullary fold. Beyond this elevated medullary fold or rim is the thickening *t. gl*, corresponding to that more opaque medial strip of the anterior extension of the Trigeminus plate to which attention has already been directed. The thickened portion of the ganglionic plate merges, in the lateral part of the field, into the thinner general area of the ganglionic lobe, little of which finds place in the figure.

On a casual inspection of fig. 76 it appears as if the marginal medullary rim or fold bounding the cephalic plate antero-laterally became, if followed backwards, directly confluent with, and continued into, the localised thickening at the base of the trigeminal lobe and in front of the transverse constriction seen in section in figs. 84 and 85. But this appearance of continuity is entirely fallacious, for, as must already have become apparent, the opacity of the two regions is the effect of quite different structural conditions. The medullary fold ends posteriorly in a tolerably abrupt fashion, whilst the small basal trigeminal ganglionic thickening behind it is really continued, not into the medullary rim, but in a forward and outward direction alongside of the medullary rim, and even partially overlapped by the free margin of the latter, as may be recognised in fig. 86.

It has been stated previously that in the slightly older Specimen M the specialised thickening in the anterior part of the basal region of the Trigeminus lobe is no longer apparent (*cf.* fig. 75). In consequence of this fact, the boundaries of the transverse encephalic constriction are in this specimen more clearly evident. The medial marginal part of the anterior extension of the trigeminal plate still shows somewhat greater opacity than the body of the plate. Fig. 87, Plate 15, represents a low-power photomicrograph of a transverse section through Specimen M in a plane a few sections

behind the posterior limit of the medullary fold, and therefore passing through the region of the encephalic constriction. In this photomicrograph the distinction between the "neural crest" cells constituting the great Trigeminus ganglionic plate or lobe, on the one hand, and the mesodermal cells underlying the medullary plate, and extending outwards in relation to the entoderm as far as the parietal cœlom, on the other hand, is tolerably clearly visible. This is especially the case in the neighbourhood of the medullary margin, and again far outwards, where the lateral portion of the ganglionic plate comes into relation with, and overlaps, the medial part of the cœlomic cleft, or "parietal cavity."

We have seen in fig. 76 how, in Specimen H, the elevated medullary rim lies horizontally; and it retains this attitude throughout its entire extent. In Specimen M, on the other hand, it assumes an oblique or even nearly vertical position, becoming at the same time concave medially. This condition is illustrated in HILL and MARTIN'S figs. 2 and 3, Plate 10. In their fig. 3 the optic groove is very evident. In their fig. 2, however, the concavity there shown is incorrectly lettered "*op. gr.*" In point of fact, this section passes wholly in front of the anterior horn of the longitudinally (or paraxially) crescentic optic groove. This anterior extremity of the optic groove really fades away insensibly into the general concavity of the up-standing medullary fold of the fore-brain region. It is this latter concavity which is wrongly designated as the optic groove in fig. 2 of HILL and MARTIN'S paper.

In front of the connection of the base of the Trigeminus ganglionic lobe with the medullary plate in the region of the fifth prefacial neuromere, no further ganglionic outgrowth or connection is met with. The sole representative of the neural crest, in front of the plane indicated, is the forward extension of the Trigeminus lobe itself, which, as we have seen, lies alongside the anterior segment of the cephalic plate, but is wholly unconnected with it.

(c) *Region of First "Prefacial" Neuromere.*

Immediately posterior to the Trigeminus ganglionic lobe there exists, as has been pointed out, a very definite break in the continuity of the cranial ganglionic masses. Opposite this marked hiatus there is differentiated in the medullary plate that most prominent of all the neuromeres, the first prefacial.

Fig. 88, Plate 15, illustrates a transverse section across the cephalic plate of Specimen M in the plane of this neuromere. The neuromeric thickening is considerable, and it is markedly convex ventrally, both in the transverse and in the cranio-caudal directions (*cf.* fig. 93).

It is quite evident from fig. 88 that there is in this region no proliferative activity at the edge of the medullary plate, and no ganglionic elements are visible anywhere in the section.

It is this first prefacial neuromere which corresponds with "Segment 8" of

CHAS. HILL ('00), as well as with "Encephalomere iv" of NEAL ('98). Neither of these observers found any ganglionic proliferation from, or connection with, this neural segment. NEAL, however, found in embryos of *Squalus acanthias* of 15–16 somites, that "a few cells with protoplasmic processes occur in the space between the neural tube and the overlying ectoderm," and he remarks that "these may indicate that at one time this encephalomere was a region of cell-proliferation and thus possessed a neural crest; but since the cells soon disappear, and since no new ones take their place, this encephalomere may be said to be a region of the neural tube which now (in *S. acanthias*) possesses no neural crest" (*loc. cit.*, p. 212).

It is, therefore, interesting to find that, in Specimen M of Ornithorhynchus, the homologous neuromere is likewise to all appearance a barren one, and that the neural crest opposite it is wholly unrepresented. On the other hand, in Specimen H, which belongs substantially to the same stage, though certainly just a shade younger, we do find a slight, but definite, representative of the neural crest connected with this first prefacial neuromere.

Fig. 89 represents a photomicrograph from a section through this neuromere, giving a high-power view of the region in the neighbourhood of the margin of the medullary plate. The transition-zone between the latter and the general ectoderm is characterised by the more irregular arrangement of its cells. From the deep aspect of this transitional region an obvious proliferative out-growth of cells is manifest. As the serial sections are followed in a caudal direction this cellular out-growth diminishes and loses its connection with the margin of the plate; but it continues to be represented by a few cells in each section, and, at the hinder limit of this neuromere, these again increase in number, becoming then continuous with the acustico-facial strand.

On the other hand, even in Specimen H there is absolutely no representative of this ganglionic rudiment opposite the anterior portion of the first prefacial neuromere; so that there the neural crest is *absolutely* interrupted.

(d) *Region of "Facial" Neuromere.*

Fig. 90, Plate 15, represents part of a transverse section through the plane of the anterior portion of the "facial" neuromere in Specimen H. Since the plane of the section is transverse to the cephalic axis, it cuts obliquely the obliquely-directed portion of the pedicle of the acustico-facial ganglion (*cf.* fig. 76). This "pedicle" is simply the basal part of the entire ganglionic cellular expansion of the "neural crest" belonging to the acustico-facial nerve-complex. The "pedicle" is narrow, relatively to the distal terminal enlargement which constitutes the ganglion proper. The figure shows the cellular continuity of the obliquely-cut pedicle ("*a-f. s.*") with the margin of the medullary plate.

The connection just referred to is not, however, an absolutely direct one. It is

easy to distinguish in the figure a marginal zone intermediate between the fully differentiated ectoderm of the medullary plate and the general ectoderm of the head.

This transitional area is obviously neural in character, and it is continuous in front with the similar, but much narrower, marginal intermediate zone visible in fig. 89. Now, it is with the deep surface of this *intermediate neural zone*, rather than with the actual edge of the definitive medullary plate, that the pedicle of the acustico-facial ganglionic Anlage is seen to be connected, in the plane here figured. But, very shortly behind this plane, the intermediate neural zone, visible in figs. 89 and 90, becomes indistinguishable, and the ganglionic connection with the edge of the medullary plate then becomes an immediate one. This condition is well seen in the photomicrograph in fig. 91, which represents a section through the same "facial" neuromere, but further back than the plane of fig. 90. (In this figure there is further evident a considerable mesial extension of ganglionic cells ("*mg.*") lying beneath the outer portion of the medullary plate.) In the plane now reached, the surface-ectoderm laterally to the medullary plate is seen to be markedly thickened and slightly depressed. This thickened patch of ectoderm forms the anterior portion of the auditory plate.

The auditory area or plaque is more adequately shown in the succeeding fig. 92, Plate 16, from a photomicrograph taken at a magnification of 175 diameters, *i.e.*, only half of the magnification of figs. 89–91. The plane of section is 0·05 millim. posterior to that of fig. 91, and passes through the posterior part of the same "facial" neuromere. Here the slightly depressed auditory plate, characterised by its thick ectoderm, is seen extending outwards from the margin of the medullary plate. Closely applied to the deep surface of the auditory plate may be seen the cellular pedicle ("*a. f. s.*") of the acustico-facial ganglion ("*a-f.*"). Medially, the pedicle may be clearly seen to become continuous with the deeper aspect of the edge of the medullary plate; whilst laterally it merges, just beyond the lateral limit of the thickened auditory plaque, in the massive acustico-facial ganglion. Owing to the marked obliquity of the comparatively narrow pedicle it is only in a very few sections that the continuity of all these structures is visible in one and the same section. Even in the one chosen for illustration there is almost an interruption between the main ganglionic mass and the "pedicle." It will be observed that the outer part of the ganglion reaches nearly as far as the medial limit of the "parietal cavity." (Here again may be noted a medially directed extension of ganglionic cells, "*mg.*," underlying the marginal portion of the medullary plate which appears in the left-hand side of the figure.)

It will appear from an examination of fig. 76 that the facial neuromere is somewhat less opaque than either the first prefacial or the first postfacial neuromere. This will also be evident from a glance at fig. 93, representing a portion of a longitudinal sagittal section through the right half of the hind-brain of Specimen H. Here the ventral surface of the "facial" neuromere, "*f. N.*," is seen to be less convex than that of either neuromere in front of or behind it; and it is also seen to form a sort of

elevated plateau, a character which is largely owing to the slight concave depression of the upper surfaces of the neuromeres fore and aft of it. These characters are not accidental to this specimen, since their existence has been confirmed by us in the case of another specimen of about the same stage as that of Specimens H and M, which was too much twisted to be generally serviceable, but which, nevertheless, in this region yielded tolerably perfect sagittal sections. Supported by this specimen we are able to affirm that fig. 93 offers an accurate picture of the organisation of this region of the cephalic plate as seen in longitudinal sagittal section.

(e) *Region of First to Third "Postfacial" Neuromeres.*

Fig. 94 represents a portion of a transverse section of the hind-brain of Specimen H, through the "first postfacial" neuromere (the same neuromere as that designated by some writers the "auditory" neuromere). This neuromere lies opposite the posterior part of the "auditory plate." In the vicinity of the junction between the latter and the edge of the medullary plate, ganglionic cell-elements are entirely absent, unless a few isolated cells clinging to the deep surfaces of the adjacent margins of these plates may possess this significance. In any case the continuity of the neural crest is practically, if not absolutely, interrupted in this region.

Laterally to the auditory plate the caudal portion of the acustico-facial ganglion still appears as a thick cell-mass ("*a-f.*"), though its continuity with the medullary plate through the ganglionic pedicle is no longer recognisable in this plane of section.

The section represented in fig. 95 cuts the cephalic plate transversely in the region of the next or second postfacial neuromere. It was previously noted in connection with this region, as shown in fig. 76, that this neuromere exhibits at its outer extremity a definite localised opacity. The explanation of this feature now appears in fig. 95, in the shape of a specialised thickening and curvature of the outer segment of this neuromere. We are unable to interpret the significance of this modification. It bears some resemblance to the process of elevation of a lateral medullary fold, but such an interpretation is hardly reconcileable with its limitation to this one neuromere. Laterally to the neuromere the hind-most part of the ectodermal thickening, which in front forms the auditory plate, is still met with in the section. But the medial edge of this thickened patch is connected with the slightly upturned margin of the neuromere by an intermediate zone of ectoderm which bends sharply dorsally in order to gain its attachment to the true border of the medullary plate. The abrupt dorsal upturning of the ectoderm by the side of the medullary plate forms a tolerably definite sulcus alongside the latter; from the deep aspect of the ectoderm forming the floor of this groove, cell-proliferation is going on and giving rise to cells. A tolerably massive and compact cellular extension outwards from this region is seen in the figure,

lettered "*g-v.*" This is the section of the thickened anterior marginal portion of the ganglionic plate or lamina representing the Glossopharyngeus-vagus nerve-complex, whose presence was indicated in connection with the course of the general description of the stage, and illustrated in fig. 76. The portion of this lamina now seen in fig. 95 is in series with and homologous to the acustico-facial pedicle, and may, in our opinion, be taken to represent the glossopharyngeal segment of the entire ganglion-complex.

There is again visible, in fig. 95, an extension of ganglionic cells ("*mg.*") in a medial direction beneath the outer segment of the medullary plate. These appear to be in series with the few cells visible in fig. 94. As the serial sections are followed backwards from the region of the first, into that of the second, postfacial neuromere, the medial ganglionic cell-group increases to the extent shown in fig. 95, whilst at the same time the lateral ganglionic expansion "*g-v.*" makes its appearance, and rapidly extends outwards in the sections.

Fig. 96 reproduces a low-power photomicrograph of one of the serial sections through the third postfacial neuromere of Specimen H. Here the vago-glossopharyngeal ganglionic plate or lamina is seen in its full transverse extension. Its outer half ("*g-v. l.*") is considerably thicker and more compact than the median half ("*g-v. m.*"). The latter forms an attenuated stratum which extends medially as far as the lateral margin of the medullary plate, where it is more or less continuous with the transitional ectoderm at the junction of the medullary plate with the general ectoderm of the head. In the section figured there is no actual connection with the edge of the medullary plate itself, but in a neighbouring plane there may be seen a slight proliferation of cells at the margin of the medullary plate, joining with the medial edge of the ganglionic plate and forming a contribution thereto. This proliferation is serially homologous with that observed at the margin of the second postfacial neuromere, which we have recognised as probably representing a glossopharyngeal segment. The proliferative connection in the region of the third postfacial neuromere is probably to be interpreted as an anterior vagal connection (Urvagus ?). It is actually visible in the original photomicrograph of which fig. 76 is a reproduction, as a minute projection at the lateral margin of the medullary plate opposite the anterior portion of the third postfacial neuromere. From this projection, especially on the right side of the photograph, a slight band of opacity may be followed outwards, indicative of a strand-like thickening of the ganglionic plate at that transverse level, and parallel with the thicker and more marked glossopharyngeal thickening in front of it, which extends outwards from the edge of the neuromere in front.

Another proliferative connection, between the vago-glossopharyngeal ganglionic plate and the margin of the medullary plate, occurs a short distance behind the plane just described, and opposite a point which would correspond to the anterior portion of a *fourth* postfacial neuromere, were such a segment clearly distinguishable at

this stage. This also is visible with the aid of a lens in the photomicrograph aforesaid, as a minute-pointed projection beyond the lateral margin of the medullary plate.

Similar minute cellular projections recur, still further caudally, at distances respectively of 0·1 and 0·15 millim., viz., throughout just that region of the myelencephalon, in which earlier indications of indefinite neuromeric segmentation were recognised.

Posteriorly to the region of the medullary plate last dealt with, the ganglionic plate thins out, and gradually becomes indistinguishable as such in section, before the neighbourhood of the first somite is reached.

The outer and more solid half of the vago-glossopharyngeal plate ("*g-v. l.*") is seen, in fig. 96, to extend so far laterally that its outer margin overlaps the inner limit of the parietal or pericardial cœlom.

(4) General Presentation and Summary of Features of Neural Organisation in the "Neurular" Stage in Ornithorhynchus.

On the basis of the foregoing facts and observations, we believe that it may be possible to frame the outline of a general conception of the neural organisation of the cephalic region in Ornithorhynchus.

In the stages above treated of, we have been dealing with a neural axis which long preserves its primitive flattened condition as a neural plate, and is thus quite uninfluenced by various factors which in other forms complicate the problem of early brain-development. For not only does the mere transformation of a flattened plate into a tube itself complicate the investigation of the early structural features, but, associated with this process, there arise a series of mesodermal differentiations which more or less directly modify and obscure the fundamental structural arrangements. Now it is hardly too much to say that here, in Ornithorhynchus, we have the spectacle of the earlier processes of neural development proceeding without any extraneous interference; and that, accordingly, we have before us, in figs. 73–76, a schematically clear representation—almost a plane construction—of a definite and important stage in the process of early neural development. In the further elucidation of this scheme, we shall practically confine our attention to its manifestation in the cephalic region. With this the spinal region falls naturally in line, demanding, for our purposes at least, no special treatment.

The Ornithorhynchus embryos, described in the later sections of this paper under the designations Z, ZZ, H, and M, all concur in revealing a condition of neural organisation, which may be summed up as follows :—

(*a*) The neural axis exists in the form of an elongated flattened plate, which, in front of the region of obvious somitic differentiation, dilates into a spathulate cephalic expansion.

(*b*) Posteriorly this cephalic expansion graduates into the spinal medullary plate, and it is impossible to place a precise limit to the transition from myelencephalon to spinal-cord-region, or myelon proper.

(*c*) Anteriorly the cephalic expansion of the neural plate exhibits a definite bilateral indentation, or constriction, which is, perhaps, the sole indication of the definitive brain-subdivision.

(*d*) On the other hand, there is observable, throughout the greater part of the cephalic plate, a definite and constant neuromeric segmentation. This segmentation is not only prior to the appearance of the definitive subdivision of the brain, with the exception above mentioned, but also, as far as can be seen, to any non-neural segmentation whatsoever, whether in the way of mesodermal cephalic segments or cavities, or of branchiomeric subdivisions.

(*e*) There is further present, alongside this segmented cephalic medullary plate, a series of ganglionic plate-like expansions, connected with the edge of the medullary plate, but extending far out beneath the general ectoderm towards the peripheral zone of the embryo.

(*f*) These ganglionic plates are three in number, and include (1) a relatively enormous Trigeminus plate or lobe, which, in the later specimens, has extended far forwards as well as outwards, by the side of the anterior portion of the medullary plate; (2) a relatively slender acustico-facial expansion, terminating distally in a very definite ganglionic enlargement, and separated from the Trigeminus plate by a very distinct hiatus; (3) a ganglionic plate, elongated considerably in the cranio-caudal direction, and representing the ganglionic rudiments of the glossopharyngeus and vagus nerves. This plate is separated from the acustico-facial by a very slight interval only, in which a few cells are still to be found which may probably represent the primitive continuity of the two ganglionic rudiments.

(*g*) The vago-glossopharyngeal plate gradually narrows and thins away in the posterior region of the myelencephalon, until it can no longer be distinguished as such in the sections.

(*h*) Behind this plane there reappears in the serial sections a spinal "neural crest," or ganglionic band, possessing the same relation to the spinal medullary plate that the ganglionic plates aforesaid do to the cephalic medullary plate, though less extensively developed than they.

(*i*) No trace of commencing differentiation of the olfactory organ is discoverable.

(*k*) Neither is any structure recognisable that could be identified with a cerebellar rudiment.

(*l*) Apart from the series of neuromeric segmental thickenings, which may possibly foreshadow the differentiation of the motor nerve nuclei, there is no indication of the presence of Anlagen of the motor nerve-roots.

(5) Antecedent Conditions of "Neurular" Organisation Illustrated from Embryonic Stages in Marsupials.

The question may now be raised as to the nature of the structural conditions which immediately precede those characteristic of the "neurula" stage which we have just been considering. Unfortunately we have not hitherto been successful in obtaining any specimen of a monotreme embryo intermediate in character between that of the "postgastrular" stage described in the earlier part of this paper and that of the fully declared "neurula." But in the postgastrular stage there was no evidence whatever of any differentiation of neural ectoderm.

In the absence of intervening stages of monotreme embryos, it will prove of the utmost interest to take note of the characters of certain stages of marsupial embryos which to some extent at least correspond to the period of development which is unrepresented in our collection of monotreme embryos.

Fig. 78, Plate 1, already referred to in the preceding pages, represents a photo-micrograph of an embryo of *Dasyurus viverrinus*, which—although it has already reached the neurular stage of development—is yet in some respects less advanced than the monotreme specimens we have been considering.

In this embryo somites are only doubtfully distinguishable. The medullary plate is differentiated, but its margins are not clearly visible throughout, on surface-view, although they are readily recognisable in section. The relative obscurity of the margins is largely due to the presence of a strong neural crest, with massive ganglionic outgrowths in the cephalic region, which give to the latter a very striking and peculiar appearance. As in Ornithorhynchus, so also here, there are huge lateral Trigeminus lobes, formed by thick sub-ectodermal cakes of cells. These obscure the surface-view of the margins of the anterior part of the cephalic plate, and even extend forwards considerably in front of it. Posteriorly, each Trigeminus plate is separated by a short interval from a tolerably massive longitudinal thickening of the neural crest which continues backwards in the posterior cephalic region. This is the continuous Anlage of the ganglia of the Acustico-facialis, Glossopharyngeus and Vagus nerves, undifferentiated as yet into its individual components. It will be observed that the cranial extremity of this Anlage is separated by only a very slight interval from the caudal end of the Trigeminus lobe. In several embryos of practically the same stage we can, in fact, recognise a slender, but distinct, longitudinal commissural connection between these two great ganglionic Anlagen. On the other hand, in a slightly later stage, a break of continuity occurs between the Acustico-facial and the Glossopharyngeal portions of the neural crest, introducing the condition which we have seen to obtain in the Ornithorhynchus stage above described.

In the Dasyurus embryo shown in fig. 78 two very distinct neuromeres are perceptible. The more anterior of these is the first prefacial. This identification

is placed beyond doubt by the examination of a number of specimens of the same and of nearly related stages. With regard to the second we are still in some doubt as to whether it represents the facial alone, or that together with the first postfacial. In either case it is noticeable that a dark band, directed obliquely outwards, connects the hinder part of this single or double neuromere with the vago-glossopharyngeal moiety of the common posterior cephalic ganglionic Anlage. It is, however, unnecessary to discuss the neuromeres and their connections in Dasyurus in the present paper. The condition in Dasyurus has merely been cited in order to illustrate that primitive continuity of the ganglionic Anlagen in the cephalic region which we infer to have existed in Ornithorhynchus prior to the discontinuous condition of the cephalic neural crest illustrated in figs. 73–76.

We are indeed in a position to carry this inquiry into precedent conditions one stage further. In our fig. 72, already referred to, there is reproduced a photomicrograph of an embryo of *Perameles obesula* which may be described as belonging to the close of the postgastrular phase. Here, on each side of the median line, there is seen a tolerably broad paraxial region, traversed by a primitive streak posteriorly, and by an archenteric axis throughout its anterior region. This area is definitely widened in the cephalic region, whilst posteriorly it tapers away into the lanceolate area traversed by the primitive streak. It is characterised both by its thick paraxial mesoderm and by its much-thickened ectoderm.

Surrounding this thickened paraxial area, and separated from it in the photograph by a narrow clear band, is the parietal embryonic zone. This is tolerably narrow and is itself again limited, peripherally, by a narrow clear band which separates it from the extra-embryonic vascular area. The latter forms a broad opaque zone, bounded by a fairly definite peripheral boundary.

The photomicrograph in fig. 72 affords no evidence of any intrinsic ectodermal differentiation within the area of thickened ectoderm covering the paraxial zone. In other words, the precise boundaries of the future medullary plate are not recognisable when the embryo is viewed as a whole. Nevertheless, in the serial sections there is no difficulty in recognising, at a distance of 0·5 millim. away from the median line (= 5 millims. in the photograph), a distinct surface-indentation marking out the edge of the definitive medullary plate from the almost equally thick ectoderm just beyond it. Towards the outer margin of the paraxial zone the latter gradually thins out into ordinary cubical ectoderm. This statement applies actually to transverse sections taken at, and just in front of, the plane of the primitive knot. The lateral limitation of the margin of the medullary plate may, however, be traced throughout the whole of the serial sections in front, right into the wide cephalic region visible in fig. 72. Here the medullary plate is considerably wider than it is posteriorly, and at the same time we find that the thick ectoderm adjoining the outer margin of the plate no longer simply fades off insensibly into thin ectoderm, but shows a second (lateral) line of demarcation at some distance

external to the edge of the medullary plate. Betweeen these two lines the ectoderm attains its maximum thickness. This is indicated in fig. 72 by the dark crescentic opacity which constitutes the most lateral, marginal, portion of the cephalic expansion of the paraxial embryonic zone. The marginal crescentic opacity is thus the optical expression of the presence of a specially thickened extra-medullary zone of ectoderm, which is transitional, or intermediate between the medullary plate and the indifferent ectoderm of the cephalic region.* The crescentic thickening is indeed simply the Anlage of the cephalic neural crest. Fig. 97 represents a photomicrograph of a transverse section through a Perameles embryo of the stage now under consideration. Here, beyond the margin of the medullary plate (*m. m. p.*), is seen the intermediate zone of thickened ectoderm (*n. ect.*). The section figured lies far forward in the cephalic region, cutting across the anterior thickened extremity of the archenteron (*cf.* figs. 69 and 71). Owing to this fact the medullary plate is narrower, and the extra-medullary intermediate zone of ectoderm is thinner relatively to the medullary plate, than would be the case a little further back, across the wider part of the cephalic expansion.

It appears clear that the extra-medullary zone here figured and described must be regarded as consisting of neural ectoderm, and as forming the ectodermal Anlage of the "neural crest," from which, at a slightly later stage, the cephalic ganglionic outgrowths already described take their origin.

As this intermediate zone of neural ectoderm is continued backwards behind the cephalic expansion, its definite peripheral limit, in this stage, gradually disappears; but it is, nevertheless, still represented by the zone of thick ectoderm which covers the lateral region of the paraxial zone beyond the still recognisable lateral margin of the medullary plate.

Proof of the correctness of the above interpretation of the condition in the early Perameles is obtainable from the study of the serial sections of the more advanced Dasyurus embryo illustrated in fig. 78. And this in turn throws the clearest possible light upon the mode of origin of the ganglionic masses in Ornithorhynchus, which it is the object of this section of our inquiry to unravel.

Fig. 98, Plate 17, represents a section through the head of such a marsupial embryo as that shown in fig. 78. The section passes about 0·13 millim. in front of the posterior extremity of the very conspicuous Trigeminus lobe seen in fig. 78. Here, in fig. 98, the margin of the medullary plate is clearly defined as an indentation on the surface (*m. m. p.*). Beyond this indentation the ectoderm extends laterally as a thickened layer as far as the median limit of the pericardial cœlom, where it rapidly thins out. Of this ectoderm, the portion immediately adjoining the medullary

* The lateral boundary of this thickened extra-medullary opaquer zone of ectoderm, in the region of the cephalic expansion, is not a perfectly continuous and regular one; and its surface is stated in our original notes to have presented an irregular and furrowed appearance. Evidence of this irregularity appears, in the serial sections, in the form of superficial notches and depressions.

plate shows, at this stage, special features. Its deep surface is completely continuous with a bulky cell-mass which is likewise continuous, medially, with the deep part of the margin of the medullary plate. This bulky cell-mass is no other than the Trigeminus ganglionic plate or lobe which, at this stage at least, is more massive than it is in our Ornithorhynchus specimens. Its deep surface reaches to the entoderm, although some mesodermal cells may possibly be squeezed between it and that layer. Laterally it extends almost as far as the limit of the "parietal" cavity, or the mesothelial lining of the latter.

Fig. 99, from the same embryo, shows a condition practically identical with the last. This section, however, passes through the acustico-facial portion of the posterior elongated ganglionic expansion visible in fig. 78. Here, again, we have a massive, though less extensive, ganglionic downgrowth from the ectoderm bordering the edge of the medullary plate, and partly continuous with the latter. This ganglionic mass also extends outwards in the direction of the parietal cavity. The figure shows very clearly the distinction of the ganglionic mass from the underlying mesoderm, and the compression of the latter sheet against the entodermal layer.

It is evident that in this marsupial embryo we are dealing with the production of ganglionic plates or lobes of essentially similar character to those already described in Ornithorhynchus. We are here able to recognise their derivation from just such an extra-medullary zone of neural ectoderm as was found to be present in the case of Perameles illustrated in figs. 72 and 97. Figs. 98 and 99 enable us further to understand how it is that the ganglionic plates met with in Ornithorhynchus become sub-ectodermal, through a process of proliferative downgrowth from an originally quite superficial narrow zone of extra-medullary neural ectoderm. It is to be noted in fig. 98 that the proliferative downgrowth of ganglionic cells is connected, not only with the extra-medullary neural ectoderm, but also with the deeper portion of the margin of the medullary plate itself.

In the light of the structural arrangements above detailed in the cases of two marsupials, the condition of the ganglionic Anlagen in Ornithorhynchus becomes luminous. In the case of each form we have to note the differentiation, from an intermediate ganglionic zone of neural ectoderm, of definite ganglionic proliferative downgrowths which are seen to extend outwards, beneath the general ectoderm into the lateral region of the head. In Dasyurus this proliferative downgrowth of neural crest-cells is more or less continuous cranio-caudally, being interrupted only between the Trigeminus and Acustico-facial Anlagen. Even in this situation, indeed, there is, in some specimens, evidence of a former continuity of the neural crest across this hiatus. In our relatively more advanced stage of Ornithorhynchus, represented by Specimens H and M, the ganglionic Anlagen have reached a still greater extension; but there is now a definite hiatus, not only between the Trigeminus and the Acustico-facialis, but also between the latter and the Glossopharyngeus-vagus ganglionic Anlage.

It has been pointed out, above, that the caudal extremity of the posterior cephalic ganglionic plate (Vago-glossopharyngeal) gradually thins out and becomes indistinguishable, as such, in our Ornithorhynchus specimens, before the region of the spinal ganglionic crest is reached. Primitively these were doubtless continuous, as they to some extent still appear to be in the case of the Dasyurus embryo. The result of the definite interruption of primitive ganglionic continuity in the positions specified, is the establishment, in the cephalic region of Ornithorhynchus, of the three ganglionic lobar expansions above described in detail, and belonging respectively to the Trigeminus, the Acustico-facialis, and the Glossopharyngeus-vagus complexes.

(6) Comparison with Conditions described in other Vertebrates.

(a) *As regards Neural Crest.*

The essential features of the condition thus defined have repeatedly been observed in vertebrate embryos. Thus, in the following passage from his 'Notes on Elasmobranch Development,' A. Sedgwick (**'92**) describes an almost precisely similar condition :—"In Scyllium and Pristiurus the nerve crest is not a continuous structure as Wijhe and Kastschenko assert (Balfour and Marshall have no observations on the cranial part of the nerve crest in Elasmobranchs). It is in three separate pieces. The first of these is found in the anterior part of the brain ; the fifth nerve and presumably the ophthalmicus profundus grow out from it. The second is found a little further back, and gives origin to the seventh and eighth nerves. The third piece occurs a little further back and reaches from the hind-brain continuously back the whole length of the spinal cord. The ninth and tenth cranial nerves and the posterior roots of all the spinal nerves grow out from it. It is this latter part of the neural crest which gives rise to the longitudinal commissure of Balfour" (p. 582).

Various degrees of continuity or discontinuity have been alleged to exist between the different cephalic ganglionic Anlagen. Thus Mitrophanow is quoted by Neal (**'98**) as stating that "at the beginning the Facialis is not wholly separated from either the Trigeminus or the Vagus group." Neal states that he himself found, on the contrary, a complete gap between the Trigeminus Anlage and the more posterior portion of the neural crest.

Again, Neal states (p. 215) that "both Rabl (**'92**) and Hoffmann (**'94**) have held that the pre-auditory portion of the neural crest is discontinuous with the postauditory portion, and Rabl considers this another proof that the pre-auditory region is one *sui generis*." "On the other hand," Neal continues, "Dohrn (**'90**) and Mitrophanow (**'93**) have stated, like the present author, that they find the crest continuous in the two regions."

Brauer (**'04**) has recently investigated the development of the cephalic neural

crest, and more particularly that of the Trigeminus ganglion, in the Gymnophiona. In his text-figures and descriptions, the differentiation from the neural crest of three ganglionic lobate expansions, much as we have shown to occur in Ornithorhynchus, becomes manifest (*cf. eg.*, his text-fig. B). Were it not for the fact of the association of this phase of ganglionic development with the uprising of medullary folds, the resemblance to the condition in the neurular stage of the Monotreme would be even more striking.

In reference to trigeminal development, BRAUER claims to demonstrate that the ophthalmic portion of the Trigeminus-ganglion complex has both a separate and a different origin from that of the maxillo-mandibular component. The former originates solely from a thickened epidermal placode, and receives no contribution from the neural crest. The latter, on the other hand, is exclusively a product of the neural crest, and receives no addition of epidermal elements.

No indication of double origin, in this sense, of the Trigeminus ganglion in Ornithorhynchus has been met with in the course of our investigations. Our sectional figs. 84 and 85 do, indeed, suggest ganglionic-epidermal connection in the probable "mesocephalic-ganglion" region. But there cannot be the slighest doubt that here we have a genuine massive outgrowth from the neural crest, forming an integral part of the trigeminal lobe.

(b) *As regards Completion of the Neural Tube.*

In this paper we have already remarked upon the advanced character of the neural and especially the ganglionic development which is attained in Ornithorhynchus prior to any curvature of the medullary plate. Nearly all the literature bearing upon the early phases of ganglionic development deals with forms in which the medullary plate either has already formed, or is in process of forming, a tubular structure. In no single case known to us has ganglionic development been worked out in connection with a perfectly flat medullary plate. As a consequence of this, the para-medullary zone of neural ectoderm, described above, has not hitherto received adequate recognition. It represents, of course, that transitional strip of ectoderm which in ordinary cases is found at the summit of each of the medullary folds, and which meets its fellow of the opposite side during the dorsal fusion of these folds. It thus practically corresponds to the neural crest of authors, from which arise the definitive ganglionic outgrowths. The appearance of this neural crest has, of course, been often recognised in stages antecedent to the closure of the medullary tube. This is the case, *e.g.*, in Selachii, according to BEARD ('88) and DOHRN ('90), and their observations have been more lately confirmed by NEAL ('98). The latter author has found (in Squalus) that "at an early stage, when the cephalic plate is still widely open, the fundament of the Trigeminus is clearly differentiated from that portion of the neural plate which is destined to form the neural tube. The

disassociation of the neural crest cells in this region, and their resultant loss of compact arrangement, have taken place to a considerable extent before the neural folds meet in the mid-dorsal line" (p. 212).

But we have seen that in the Monotreme (and so also in Marsupials) the differentiation of the neural crest and its ganglionic derivatives from para-medullary neural ectoderm, precedes the appearance of medullary folds, or of any curvature whatever of the neural plate itself.

(c) *As regards the Supervention of Neuromeric Segmentation.*

It is beyond our purpose, in the present paper, to undertake the discussion of the entire problem of neural segmentation; nor do we propose to attempt a systematic review of the literature bearing upon this question. A tolerably complete account of the subject, up to the date of his paper, has been furnished by H. V. NEAL ('98), who also appends a full bibliography.

The well-nigh schematic clearness of the neuromeric differentiation in the cephalic plate of Ornithorhynchus absolves us from the necessity for any lengthy discussion of criteria employed in their recognition. We are, however, disposed to agree with NEAL in his remark (*loc. cit.*, p. 252) that "local thickening" is a more essential characteristic of a hind-brain neuromere than the commonly accepted criteria of a radial arrangement of cells in the neuromere, and the crowding of the cells in the zones of constriction between neuromeres. These latter characters are, nevertheless, not wholly absent in the case of the neuromeres in Ornithorhynchus, in spite of the fact that here we cannot trace the operation of any *extrinsic* "mechanical influences" such as NEAL imagines may be the factors in determining such nuclear arrangements.

According to NEAL, the best criteria to test the neuromeric value of any given division of the neural tube are "such as associate the supposed neuromeres metamerically with other structures known to be segmental, *e.g.*, the mesodermic somites or the segmental nerves." In Ornithorhynchus, however, at the stages examined by us, there is an entire absence of every trace of segmentation in the very scanty mesoderm of the head, so that there can certainly be no dependence of neuromeric segmentation on the segmentation of the mesoderm in this case. And, in view of the association of the Trigeminus ganglionic Anlage with a series of neuromeres, it is obviously impossible to apply the test of association with a segmental nerve to the determination of such a question as, *e.g.*, the genuine neuromeric value of the apparent duplication of our "third prefacial" neuromere.

W. A. LOCY had, previously to NEAL, published important contributions to our knowledge of the structure and development of the vertebrate head. According to this author ('95), the margins of the "cephalic plate," as well as the "marginal bands" of the blastoderm in the trunk region, exhibit an early, segmental, bead-like differentiation. LOCY claims that those bead-like segments "once established in this

very early stage, may be traced onward in an unbroken continuity until they become the neuromeres of other observers, and sustain definite relations to the spinal and cranial nerves."

NEAL (*loc. cit.*) has vigorously opposed this view, maintaining that these segmental marginal thickenings are irregular, asymmetrical, and transient, and are confined to the marginal bands, whilst he holds, in direct contradiction to LOCY, that they "cannot be traced into the 'neuromeres' of the later stages."

More recently, CHARLES HILL ('00) has strongly supported LOCY'S statements on the basis of his observations on Teleosts and on the chick. HILL'S illustrative drawings certainly strongly support LOCY'S views, but we cannot admit that HILL has fully and adequately met NEAL'S objections to LOCY'S interpretation of the early crenation of the margin of the cephalic plate, *e.g.*, in Squalus. The weightiest part of NEAL'S contention, as it appears to us, is not merely negative, as C. HILL represents it, but resides in the positive statement that the beaded thickenings found are not only asymmetrical, but are quite variable in different specimens.

Upon the controversial point at issue, we do not venture to decide. But we may remark, with reference to the primary marginal segmentation, that even LOCY would appear to admit that it is not strictly and distinctively a medullary-plate-segmentation, but one which includes the ganglion-ridges. He says (*loc. cit.*, p. 542) that "we may speak of the neuromeres as including the segments of the ganglion-ridge." And in the embryos of Squalus, which he figures in figs. 25–30, it is plain that the cephalic plate must be regarded as including both the medullary wall of the future neural tube and the lateral ganglionic ridges of neural ectoderm. It would appear to be in the latter that the primitive segmentation illustrated by LOCY first sets in, and we therefore understand him to hold that the process of neuromeric segmentation precedes the differentiation of the medullary plate proper (*i.e.*, the wall of the neural tube) from the ganglionic ridges; and also that segmentation of the latter is continuous with the neuromeric segmentation of the former. The evidence in support of the opinion, that the primitive segmentation of the margin of the cephalic plate (which is certainly ganglionic) is entirely congruous with, or is even actually prolonged medially as, a genuine neuromeric segmentation of the medullary plate proper, seems to us not wholly convincing. According to NEAL (*loc. cit.*, p. 160), the beaded marginal segments described by LOCY "are confined to the marginal bands, and therefore do not extend into the median medullary plate." And he also states that "an examination of cross-sections of the cephalic plate (Plate 7, figs. 55 and 56, '98), before the edges have fused dorsally to form a closed tube, shows that the neural crest is already differentiated from the tissue which will form the walls of the neural tube; it is differentiated as a region of rapid cell proliferation, and of less compactly arranged nuclei. If the centres of cell proliferation were fixed, then we should have a segmented neural ridge, as affirmed by BEARD ('88)."

But, however the case may be in lower vertebrates, there can be little doubt that,

in the case of Ornithorhynchus, the neuromeres belong solely to the medullary plate, *sensu stricto;* we can in no sense speak of them, with Locy, as "including the segments of the ganglion ridge." We cannot, indeed, absolutely exclude the possibility that, in some earlier stage, the paramedullary neural ectoderm, from which the ganglionic Anlagen are derived, may have exhibited segmental thickenings. But no evidence for such a supposition is obtainable from the examination of the early marsupial embryo, in which we have been able to demonstrate the earliest phase of the differentiation of the paramedullary strip of neural ectoderm, representing the neural crest. So far as our observations on monotremes and marsupials extend, the neuromeres, which are so clearly and definitely differentiated in these forms, are strictly and exclusively medullary, and are quite distinct and independent, both in their origin and in their serial arrangement, from the ganglionic segments of the neural crest.

On the other hand, we do find, as has already been shown in the preceding pages, that a series of proliferative cell-connections are established between various neuromeres and the cephalic ganglionic plates arising from the paramedullary neural ectoderm. Indeed, it must be noted that, in those cases in which the mode of origin of a ganglionic outgrowth of the neural crest is clearly to be distinguished, there is demonstrable a definite connection of the ganglionic proliferative outgrowth with the margin of the medullary plate, as well as with paramedullary neural ectoderm, from which it primarily arises. And, later on, when the ganglionic Anlage is established as a wholly subectodermal plate or mass, it loses all connection with the overlying ectoderm, but it retains a more or less intimate connection with the deep edge of the medullary plate. And from each of the neuromeric thickenings of the medullary plate, a proliferative contribution of cells seems to pass over into the neighbouring ganglionic Anlage.

(d) *Homologies of Neuromeres in Ornithorhynchus.*

It is needful to consider, though unnecessary to discuss at any great length, the question of the correspondence of the neuromeres of Ornithorhynchus with those observed in other forms.

The interpretations given by various observers to the results of their respective investigations of the neural segments in the various forms hitherto examined, have been tabulated by Neal (*loc. cit.*) on Tables 1 and 2, pp. 151 and 152. His own, in some respects rather divergent, views of the segmentation in Squalus are also conveyed in tabular form in Table 3, p. 253. Chas. Hill's more recent conclusions ('00) regarding the neuromeric segments in Teleosts and the chick, do not widely differ from those of Locy ('95) in Squalus.

Our knowledge of the cephalic neural segments in mammals is by no means extensive, as a glance at Neal's table will readily show. Nor has much been added

since the date of his paper. And when one proceeds to analyse the actual content of the communications there referred to, it is found to be but meagre. Except in marsupials and monotremes it would appear that neuromeric segmentation, in mammals as in the majority of vertebrates, does not appear until a period when its manifestation is complicated and modified, and to some extent masked, by other developmental processes. These are, chiefly, the uprising of the medullary folds and the completion of the neural tube; the appearance of the definitive encephalic plicæ and flexures; the differentiation of the more or less rudimentary head-somites, and the appearance of the Anlagen of the great cephalic sense-organs. Now, all of these processes are relatively long delayed in the case of the marsupials and monotremes, or it may be more expressive to say that the neuromeric segmentation sets in precociously. There are no published observations with regard to neuromeres in marsupials, so far as the writers are aware. It is true that several of SELENKA's ('86 and '87) figures show certain of the hind-brain neuromeres in Didelphys, but SELENKA entirely mistook their structural character and significance, referring to them as mesodermal somites, as HILL and MARTIN have already pointed out. The previous description of the neuromeres in Ornithorhynchus by HILL and MARTIN has evidently been unknown to NEAL, since he omits to include a reference to Ornithorhynchus in his Table 1, as one of the mammals in which encephalic segments have been described.

HILL and MARTIN's former description of the "Specimen M" of this paper must now, however, be pronounced to be somewhat incomplete and inaccurate as regards the neuromeres. Their entire conception of the neural organisation of the head was vitiated by the failure to recognise the ectodermal and neural character of the Trigeminus lobe. This general point of view naturally led to the determination of that segment of the medullary plate lying in front of our "first prefacial" neuromere, and behind the well-marked encephalic constriction, as "mid-brain." But, as has already been stated, this is really the anterior portion of the hind-brain, and it contains at least three pairs of neuromeres and not only one pair as stated by HILL and MARTIN (*loc. cit.*, p. 53, their single pair of "mid-brain" neuromeres). In this matter they were misled by the absence of any very obvious thickenings of the medullary plate in the region between their foremost "hind-brain" neuromere and their single "mid-brain" neuromere (*cf.* their fig. 16). Neuromeric thickenings, nevertheless, exist, though not very distinctly, in every section; and the original photomicrographs which are reproduced in figs. 75 and 76 leave no doubt of the objectivity of the segments in question.

In determining the serial homologies of the neuromeres of Ornithorhynchus with those of other forms, we have chosen the "facial" neuromere as our point of departure in the enumeration of the neural segments, for reasons which have already been adduced (p. 119). We believe that the homology of certain of these segments may be regarded as perfectly definite and conclusive.

Reference to NEAL's Table 2, on p. 152 of his paper ('98) will show that practical unanimity prevails on the subject of the association of one neuromere with the acustico-facial nerve-complex. This particular neuromere is tabulated by that author as the fourth of the hind-brain neuromeres usually recognised. It is further determined as being that neuromere which lies immediately in front of the one which lies opposite the central part of the auditory plate. These are the characteristics of our "facial" neuromere, which is therefore the one which is commonly enumerated as the "fourth hind-brain neuromere." But it is also identical with LOCY's and CHAS. HILL's ninth neural segment, and NEAL's "Encephalomere v."

The neuromere in front of that just identified, likewise possesses easily recognisable features. It is distinguished by out-standing prominence and sharpness of definition, and also by the absence, either complete or very nearly complete, of any proliferative contribution to, or connection with, the neural crest. It lies, in fact, opposite the hiatus in the neural crest which separates the acustico-facial from the trigeminal Anlagen. This, our "first prefacial" neuromere, is thus, both relatively from its position, and absolutely through its intrinsic characters, identifiable with the "eighth segment" of LOCY and CHAS. HILL, and with "Encephalomere iv" of NEAL. It appears in NEAL's Table 2 as Segment No. 3 of the hind-brain.

The "first postfacial neuromere" of Ornithorhynchus lies opposite the centre of the auditory plate. This is LOCY's and CHAS. HILL's tenth segment, and NEAL's "Encephalomere vi." It has no certain ganglionic connection, although we have stated above that a few cells persist at its margin and thus represent the otherwise interrupted neural crest at this transverse plane. So far we are unable to confirm NEAL's judgment in reference to Squalus, that the glossopharyngeus nerve is originally connected with this neuromere but is dislocated backwards in consequence of a caudal migration of the auditory plate. We cannot, however, exclude the possibility that, primitively, the anterior part of the ganglionic plate of the glossopharyngeus-vagus complex was actually connected with the medullary plate in the region of this neuromere. But we are not disposed to attach any great importance to such an early connection, believing as we do, that the definitive neuromeric segmentation sets in independently of, and perhaps subsequently to, the differentiation of the ganglionic Anlagen of the neural crest; and the formative nidus for the latter is, mainly at least, paramedullary. We have previously seen that in the neurular stage of Ornithorhynchus represented by Specimens H and M, the majority of the neuromeres have either retained, or have developed, a proliferative connection with those portions of the ganglionic Anlagen which lie opposite them. The most anterior of these connections with the ganglionic vago-glassopharyngeal plate is not with the "first," but with the "second postfacial" neuromere. It is this which we therefore regard and designate as the glossopharyngeal segment of the medullary plate. It corresponds with "Encephalomere vii" of NEAL and to the eleventh segment of LOCY and HILL.

The last neuromere which is clearly defined in the hind-brain region of Ornithorhynchus in the stages now under notice is that which we have named the "third-postfacial." This obviously corresponds to the "seventh hind-brain neuromere" (*auct.*). It corresponds with LOCY'S twelfth or anterior vagus segment, and would appear to answer to NEAL'S eighth neuromere. This author avoids the application of the term "Encephalomere" to the last four neuromeres included in the head region by annexation from the spinal. It is doubtful whether he regards them simply as myelomeres, though it is probable that he does. In Ornithorhynchus, at all events, there is not the slightest ground for distinguishing between the second and third postfacial neuromeres, whether as regards origin, structural character, or significance; and in each case their appearance is certainly independent of any extrinsic factor, mesodermal or other.

We have also seen reason to believe (*v. supra.*, p. 120), that in Ornithorhynchus the tendency to neuromeric segmentation manifests itself, at an early period, in the tapering myelencephalic region, behind the third postfacial neuromere, but in front of the first somite. Indefinite indications of neuromeres in such a situation were perceptible in the earlier neurular phase represented by our Specimens Z and ZZ. These apparently do not attain to full expression and disappear in the later stage.

It is when we turn to that region of the medullary plate which lies in front of the first prefacial neuromere, that we encounter most difficulty in homologising the neuromeres of Ornithorhynchus with those of other animals.

We have already stated our conviction that the transverse constriction of the cephalic plate of Ornithorhynchus to which we have drawn attention, corresponds more or less closely with the site of the future isthmus or region of demarcation between rhombencephalon and mesencephalon. We would, however, avoid the suggestion that the earlier and the later boundaries are really morphologically coincident. Rather does it seem probable to us that, in the stages of monotreme development at present under our consideration, we have before us in tolerably clear and uncomplicated form, an illustration of that fundamental and primitive organisation of the encephalon into the two primitive divisions recognised by v. KUPFFER, and denominated by him as "Archencephalon" and "Deutero-encephalon" respectively. In the account offered by this author of this most primitive phase of organisation of a craniate brain, there is assumed a partial or total closure of the medullary tube, so that the divisions referred to are more or less vesicular in character. Here, in the monotreme brain, we believe that the same primitive divisions may be recognised, prior to the appearance of brain-vesicles altogether.

If this homology be accepted, then the constriction in the cephalic plate of Ornithorhynchus should correspond in position to v. KUPFFER'S "plica encephali ventralis," although, in the more typical craniate brain, this latter fold appears as if it owed its existence largely to the sinking, or sagging downwards, of the floor of the "Archencephalon"; whilst in the monotreme stages dealt with there is no trace

of any such process. Further, the entire absence of any evidence as yet of a commencing flexure of the brain-plate excludes the interpretation of the transverse encephalic constriction in the monotreme as the equivalent of the kink ("Knickung") which separates the two brain-divisions named by His "Grosshirnarm" and "Rautenhirnarm." (According to v. KUPFFER the "Grosshirnarm" of His includes rather more than the "Archencephalon," inasmuch as the definitive mid-brain takes in, in the course of its formation, a small portion of the "Deutero-encephalon.") The production of the transverse encephalic constriction in Ornithorhynchus plainly owes nothing to any extrinsic mechanical factors. We are therefore disposed to regard it as a purely encephalic feature, marking the limit of v. KUPFFER's two divisions, "Archencephalon" and "Deutero-encephalon." How far its apparent destiny, as the forerunner of the future isthmus-region, involves its identity with a "plica rhombo-mesencephalica" rather than with a "plica encephali ventralis," we cannot now determine. Its early and primitive character strongly suggest its correspondence with the more primitive of the two plicæ described by v. KUPFFER. But in any case there can remain no doubt that practically the entire cephalic plate, as visible in the present stages behind the transverse encephalic constriction in Ornithorhynchus, becomes converted into hind-brain.

Now, in that region of the cephalic plate in Ornithorhynchus which lies in front of the first-prefacial neuromere, but behind the constriction aforesaid, we have seen that three, or even possibly four, pairs of neuromeres are distinguishable. Each of these is connected with the broad base of the subectodermal, plate-like, expansion which constitutes a trigeminal lobe.

At, or in front of the constriction, again, lies another pair of neuromeres, also connected by a proliferative cellular connection with the anterior margin of the base of the trigeminal lobe.

No previous writer has described more than two pairs of hind-brain neuromeres in front of our first prefacial neuromere. The neuromeric relations and connections of the Trigeminus have been variously stated. All descriptions concur in the recognition of its connection with a neuromere next in front of the easily identified first-prefacial of our classification. BÉRANECK ('87) in the chick, FRORIEP ('92) in the mole, and BROMAN ('95) in man, all allege the existence of a connection between the Trigeminus and our first-prefacial neuromere itself. Such a connection is undoubtedly lacking in our specimens of Ornithorhynchus, and the generally recognised absence of nerve-connection has in fact earned for this neuromere the designation of "nervenlos" from v. KUPFFER (*loc. cit.*, p. 264). Whilst emphasising its barren character, NEAL, however, remarks that "late in its development the fibres of the sensor root of the Trigeminus connect with its convexity" (*loc. cit.*, p 248) in Squalus. A persistent commissural portion of a neural crest continuous, in front, with the Trigeminus Anlage, may, in some cases, have simulated an actual trigeminal connection with this neuromere.

LOCY and HILL maintain the existence of a nerve-connection between the "anterior

root of the Trigeminus" and their "sixth neuromere" which answers to our third prefacial. NEAL'S views on the connections of the Trigeminus call for special remark. His "Encephalomere iv" answers to our first-prefacial. As just stated, he finds this to be secondarily connected with the Trigeminus, although he holds that no ganglionic nerve Anlage is proliferated from it. In the hind-brain, in front of "Encephalomere iv," he finds only one neural segment, viz., his "Encephalomere iii." He holds that from this are proliferated "neural-crest-cells" which "pass ventrally into the mandibular arch." This author's "Encephalomere ii" he identifies with the entire mid-brain, and states that from its simple dorsal expansion "are proliferated cells which pass ventrally and fuse with the skin to form the mesocephalic ganglion lateral to the first somite. Although this ganglion never becomes connected with the mid-brain (Encephalomere ii), since its fibres enter the brain through the ram. oph. prof. V, it must, in my opinion, be regarded as a segmental ganglion comparable with those of the following cranial nerves: the oph. prof. must likewise be considered as a dorsal nerve homodynamous with the succeeding cranial nerves" (p. 258, '98).

NEAL further states that "Encephalomere iii" divides into two; so that, according to his observations, the Trigeminus is primarily connected with the representatives of three neural segments, the anterior of which belongs to the mid-brain. This latter connection should correspond to that between the Trigeminus lobe and the fifth prefacial neuromere in Ornithorhynchus (the doubtfully duplicated third prefacial neuromere being always reckoned as one only). We have thus in Ornithorhynchus three hind-brain neuromeres in evident proliferative connection with the Trigeminus lobe as against a maximum of two hind-brain neuromeres in any other recorded form, whilst in the great majority of instances only one hind-brain neuromere is described as connected with the Trigeminus Anlage. The region of the hind-brain structurally associated with the Trigeminus lobe in Ornithorhynchus, is thus an unprecedently elongated one. Yet a comparison of fig. 78 with figs. 75 and 76, Plate 1, will serve to suggest that, in marsupials at least, the neuromeric relations of the Trigeminus lobe are probably not very widely dissimilar from those which obtain in monotremes.

The absence of any cerebellar rudiment has been noted earlier in the paper. LOCY and HILL have identified their "sixth segment" as the "cerebellar neuromere.' That segment is probably homologous with our "third prefacial." Cerebellar differentiation may, perhaps, occur later in the region corresponding to this segment. It is difficult to believe that the neuromeric thickening, as such, has anything to do with cerebellar differentiation. In any case, in the stages of monotreme development dealt with in this paper, there is as yet not a trace of anything which could be regarded as a cerebellar Anlage.

[ADDENDUM.—Since the present paper was written there has appeared a lengthy discussion by J. B. JOHNSTON ('05) on the subject of the "Morphology of the Head,"

in which the question of neural segmentation is dealt with. Confirmed by certain observations of his own on amphibian embryos, the author accepts the results of the work of LOCY and HILL as representing the present state of knowledge of the neuromeres. It is therefore hardly necessary to do more than refer here to JOHNSTON'S paper, as containing a detailed account of the morphology of the head, worked out, so far as neural segmentation is concerned, on the basis of the scheme of LOCY and HILL.]

(7) SUMMARY OF THE RELATIONS OF THE NEUROMERES IN ORNITHORHYNCHUS TO THE GANGLIONIC ANLAGEN.

The relations of the several neuromeres in Ornithorhynchus to the different ganglionic Anlagen may now be summed up as follows:—

(*a*) No fewer than four neuromeres (perhaps five, if the third prefacial neuromere is really doubled) are connected with the Trigeminus ganglionic plate.

(*b*) The most anterior of these probably belongs to the future mesencephalon. It is very likely that it corresponds to NEAL'S "Encephalomere ii," which, according to that writer, proliferates cells forming (in Squalus) the "mesocephalic ganglion" connected with the "ophthalmicus profundus."

(*c*) The "facial" neuromere is in connection laterally with the narrower medial portion, or "pedicle," of the acustico-facial ganglionic expansion. This connection is mediated by a less differentiated cellular strip of neural ectoderm.

(*d*) The vago-glossopharyngeal ganglionic plate is connected laterally with the series of "postfacial" neuromeres with the exception of the first.

(*e*) The number of these appears to vary at different periods of development. Either one or two at least seem to disappear during the course of the evolution of the "neurular" stage. In the more advanced specimens the second and third are the only recognisable neuromeres behind the first.

(*f*) Behind the plane of the latter, however, there are small cellular proliferations from the edges of the medullary plate, in that region in which more posterior neuromeres were formerly present.

(*g*) Through these cellular proliferations a faint connection of the medullary plate with the vago-glossopharyngeal plate is maintained.

(*h*) The "first prefacial" neuromere lies opposite the gap between the trigeminal and the acustico-facial ganglionic plates and has no connection with either.

(*i*) The "first postfacial" neuromere lies opposite the smaller gap between the acustico-facial and vago-glossopharyngeal plates, and also seems to be unconnected with either of these.

(*k*) The latter neuromere lies opposite the posterior, major, portion of the auditory plate, but no connection can be demonstrated as existing between them.

LIST OF REFERENCES.

('01) BALLOWITZ, E. "Die Gastrulation bei der Ringelnatter, etc.," 'Zeitsch. f. wiss. Zool.,' vol. 70, pp. 675–730, Plates xxix–xxxiii and 41 text-figs.

('88) BEARD, J. "The Development of the Peripheral Nervous System of Vertebrates," 'Quart. Journ. Micr. Sci.,' vol. 29, pp. 153–227, Plates 16–21.

('88) VAN BENEDEN, E. "Untersuchungen über die Blätterbildung, den Chordakanal und die Gastrulation bei den Säugethieren," 'Anatomischer Anzeiger,' vol. 3, p. 709.

('99) *Idem.* "Recherches sur les premiers stades du développement du Murin (*Vespertilio murinus*)," 'Anatomischer Anzeiger,' vol. 16, p. 305.

('87) BÉRANECK, E. "Étude sur les replis médullaires de poulet," 'Recueil Zool. Suisse,' vol. 4, No. 2, pp. 305–364, Plate 14.

('84) BONNET, R. "Beiträge zur Embryologie der Wiederkäuer, gewonnen am Schafe," 'Arch. f. Anat. u. Physiol.,' Anat. Abt., p. 170 (continued *ibid.*, '89).

('97) *Idem.* 'Beiträge zur Embryologie des Hundes,' Anatomische Hefte, vol. 9, Parts 28–30, p. 421.

('01) *Idem. Ibid.* 'Erste Fortsetzung,' Anatomische Hefte, vol. 16, Part 51, p. 233.

('04) BRAUER, A. "Beiträge zur Kenntniss der Entwickelung und Anat. der Gymnophionen. IV. Die Entwickelung der beiden Trigeminus-Ganglien," 'Zool. Jahrb. Suppl.,' vol. 7, pp. 381–408, Plates 21 and 22 and 7 text-figs.

('95) BROMAN, I. "Beschreibung eines menschl. Embryos von beinahe 3 mm. Länge, etc.," 'Morphol. Arbeiten,' vol. 5, Part 2, pp. 169–206, Plates 9 and 10 and 7 text-figs.

('87) CALDWELL, W. H. "The Embryology of Monotremata and Marsupialia," Part I, 'Phil. Trans.,' vol. 178 B.

('84) *Idem.* "On the Development of the Monotremes and Ceratodus," 'Roy. Soc. of N.S.W. Journ. and Proc.,' vol. 18.

('88) CARIUS, F. 'Ueber die Entwickelung der Chorda und der primitiven Rachenhaut bei Meerschweinchen und Kaninchen,' Inaug. Diss., Marburg.

('96) DAVENPORT, G. C. "The Primitive Streak and Notochordal Canal in Chelonia," Radcliffe College Monograph, No. 8, 'Contributions from the Zool. Lab. of the Museum of Compar. Zool., Harvard,' No. 67.

('99) DENDY, A. "Outlines of the Development of the Tuatara (*Sphenodon* (*Hatteria*) *punctatum*," 'Quart. Journ. Micr. Sci.,' vol. 42, p. 1.

('60) DOHRN, A. "Bemerkungen über den neuesten Versuch einer Lösung des Wirbeltierkopf-Problems," 'Anatomischer Anzeiger,' vol. 5, pp. 53–64 and 78–85.

('92) FRORIEP, A. "Zur Frage der sogenannten Neuromerie," 'Verhandl. Anat. Gesellsch.,' pp. 162–167.

('03) HERTWIG, O. "Die Lehre von den Keimblättern," 'Handbuch der vergl. u. experim. Entwickelungslehre der Wirbeltiere,' Lief. 12–13 and 14–15.

('00) HILL, CHAS. "The Developmental History of the Primary Segments of the Vertebrate Head," 'Zool. Jahrb.,' Abt. f. Anat. u. Ont., vol. 13, Part 3.

('94) HILL, J. P., and MARTIN, C. J. "On a Platypus Embryo from the Intra-uterine Egg," 'Proc. Linn. Soc. N.S.W.,' vol. 10, 2nd Series.

('94) HOFFMANN, C. K. "Zur Entwickelungsgeschichte des Selachierkopfes," 'Anat. Anz.,' vol. 9, pp. 638–653.

('90) HUBRECHT, A. A. W. "Studies in Mammalian Embryology, II. The Development of the Germinal Layers in *Sorex vulgaris*," 'Quart. Journ. Micr. Sci.,' vol. 31.

('02) *Idem.* "Furchung und Keimblattbildung bei Tarsius spectrum," 'Verh. d. kgl. Akad. Amsterdam,' vol. 8.

('05) JOHNSTON, J. B. "The Morphology of the Vertebrate Head, etc.," 'Journ. Comp. Neurol,' vol. 15, p. 175.

('93) KEIBEL, F. "Studien zur Entwickelungsgeschichte des Schweines," 'Morpholog. Arbeiten,' vol. 3.

('95) *Idem. Ibid.*, II, 'Morphologische Arbeiten,' vol. 5.

('01) *Idem.* "Die Gastrulation und die Keimblattbildung der Wirbeltiere," 'Ergebnisse der Anatomie und Entwickelungsgeschichte' (MERKEL u. BONNET), vol. 10.

('82) v. KÖLLIKER, A. "Die Entwickelung der Keimblätter des Kaninchens," 'Festschrift, Universität Würzburg,' Leipzig, 1882.

('03) v. KUPFFER, K. "Die Morphogenie des Centralnervensystems," 'Handbuch der vergl. u. experim. Entwickelungslehre der Wirbeltiere,' Kapitel 8.

('84) LIEBERKÜHN, N. "Ueber die Chorda bei Säugetieren," 'Arch. f. Anat. u. Phys.,' Anat. Abt., 1882 u. 1884.

('95) LOCY, W. A. "Contribution to the Structure and Development of the Vertebrate Head," 'Journ of Morphology,' vol. 11, No. 3.

('91) MEHNERT, E. "Gastrulation u. Keimblätterbildung der *Emys lutaria taurica*," 'Morphologische Arbeiten,' vol. 1.

('93) MITROPHANOW, P. J. "Étude embryogénique sur les Sélaciens," 'Arch. Zool. Exp.,' 3rd Series, vol. 1, pp. 161–220, Plates 9–14.

('02) *Idem.* "Beiträge zur Entwickelung der Wasservögel," 'Zeitschr. f. wiss. Zool.,' vol. 71.

('91) MITSUKURI, K. "Further Studies on the Formation of the Germinal Layers in Chelonia," 'Journ. Coll. Sci. Imp. Univ. Japan,' vol. 5, Part 1.

('93) *Idem.* "On the Process of Gastrulation in Chelonia," 'Journ. Coll. Sci. Imp. Univ. Japan,' vol. 6, Part 4.

('95) McClure, C. F. W. "Segmentation of the primitive Vertebrate Brain," 'Journ. of Morphology,' vol. 4.

('98) Neal, H. V. "The Segmentation of the Nervous System in *Squalus acanthias*," 'Bull. Mus. Compar. Zool. Harvard," vol. 31, No. 7.

('87) Orr, H. "Embryology of the Lizard," 'Journ. of Morphology,' vol. 1, p. 311.

('92) Rabl, C. "Ueber die Metamerie des Wirbeltierkopfes," 'Verh. Anat. Gesellsch.,' pp. 104–135.

('92) Sedgwick, A. "Notes on Elasmobranch Development," 'Quart. Journ. Micr. Sci.,' vol. 37, pp. 87–101.

('86–7) Selenka, E. "Das Opossum" (Wiesbaden, 1886–7).

('94) Semon, R. "Zur Entwickelungsgeschichte der Monotremen," 'Zool, Forschungsreisen in Australien, etc.,' vol. 2, Lief. 1.

('88) v. Spee, F. (Graf). "Ueber die Entwickelungsvorgänge vom Knoten aus im Säugetierkeimscheiben," 'Anatomischer Anzeiger,' vol. 3.

('92) Waters, B. H. "Primitive Segmentation of the Vertebrate Brain," 'Quart. Journ. Micr. Sci.,' vol. 33, pp. 457–475, Plate 28.

('92) Will, L. "Beiträge zur Entwickelungsgeschichte der Reptilien: (1) Die Anlage der Keimblätter beim Gecko (Platydactylus)," 'Zoolog. Jahrb.,' Abt. f. Anat. u. Ont., vol. 6.

('95) *Idem.* "Die Anlage der Keimblätter bei der Eidechse (Lacerta)," *ibid.*, vol. 9.

('03) Wilson, J. T., and Hill, J. P. "Primitive Knot and Early Gastrulation Cavity coexisting with independent Primitive Streak in Ornithorhynchus," 'Proc. Roy. Soc. London,' vol. 71.

DESCRIPTION OF PLATES.

All figures are from specimens of Ornithorhynchus unless otherwise indicated.

Drawings were executed with the aid of the camera lucida, except fig. 49, which was drawn from a photograph of the wax-plate model.

The following is a list of reference-letters common to various figures:—

a. c. = pericardial or parietal cœlom.
a-f. = acustico-facial ganglion or ganglionic plate.
a-f. s. = pedicle of acustico-facial ganglion.
alb. = layer or remains of albumen.
a. l. bl. = anterior lip of blastopore.
all. ent. = allantoic entoderm.
a. o. = area opaca.

a. o. p. = junction of entoderm of areæ opaca and pellucida.
a. p. = area pellucida.
arch. = archenteron.
arch. ps. = protosomite.
ar. c. = archenteric cavity.
ar. pl. = archenteric plate.
aud. = auditory plate.

bl. = blastoporic aperture.

ch. a. = chorda-Anlage.
cœ. = cœlom.
c. c. z. = central cellular zone of primitive knot.
c. p. = cell-plug in primitive knot of Specimen Y.
c. w. = cellular wall of archenteric cavity in Specimen DD.

d. fr. = median sulcus (Rückenfurche) of medullary plate.

ect. = ectoderm.
ent. = entoderm.

f. N. = "facial" neuromere.

gl. = neural crest or ridge.
g-v. = vago-glossopharyngeal ganglionic plate.
g-v. l., *g-v. m.* = lateral and medial portions, respectively, of vago-glossopharyngeal ganglionic plate.

h. a. = heart-Anlage.
ht. end. = endothelial heart.

i. c. m. = intermediate cell mass.

mes. = mesoderm.
mes. ax., *mes. l.* = paraxial and parietal regions, respectively, of mesodermal sheet.
mg. = medial ganglionic strand.
m. m. p. = margin of medullary plate.
m. p. = medullary plate.
m. s. = mesodermal somite.
m. s. c. = cavity of somite.
m. z. = marginal or cortical zone of primitive knot in Specimen Y.

n. ect. = neural ectoderm.

op. = opening of archenteric lumen into cavity of blastodermic vesicle.
op. gr. = optic groove.

po. N$^{1-3}$. = first to third postfacial neuromere.
pr. N$^{1-5}$. = first to fifth prefacial neuromere.
p. l. bl. = posterior lip of blastopore.
p. p. = "protochordal plate" expansion of archenteric plate.
p. pr. k. = posterior undifferentiated portion of primitive knot.
pr. g. = primitive groove.
pr. k. = primitive knot or archenteric knot.
pr. s. = primitive streak.
pr. z. = protangioblastic zone.

r. = reticulum of degenerate cell-material beneath archenteric plate.

sh. = shell.
som. = somatic mesoderm.
spl. = splanchnic mesoderm.

t. gl. = trigeminal ganglionic proliferation.
t. l. = trigeminal ganglionic plate or lobe.

vas. c. = vaso-formative cellular tissue.
v. m. = vitelline membrane.
vw. arch. = ventral archenteric wall.

w. y. = white yolk.

y. y. = yellow yolk.
y-m. = yolk-mass.

SPECIAL DESCRIPTION OF FIGURES, PLATES 1–17.

Fig. 1.—Photomicrograph [× 10 diameters] of an intact egg of 5·5 millims. diameter cleared in cedar oil. (Specimen A.) The somewhat shrunken spheroidal yolk-mass, *y-m.*, is clearly visible through the enclosing shell and vitelline membrane, *sh.* Towards the centre of the yolk-mass the germinal area is somewhat obscurely visible. This shows the first eight blastomeres arranged in definitely bilaterally symmetrical fashion.

Fig. 2.—Drawing [× 220] of vertical section through central portion of germinal disc with white-yolk-bed in a segmenting egg of about 5 millims. diameter. (Specimen NN.) Around the nuclei are seen the clear, somewhat too sharply delimited, cytoplasmic zones. The peripheral zones of the incompletely segmented cell-bodies are formed of coarsely granular deutoplasmic material, that of adjacent cells being more or less confluent.

This material is partially differentiated, deeply, from the underlying white yolk.

Fig. 3.—Photomicrograph [× 170] of part of section through peripheral region of germinal disc of same egg. (Specimen NN.) Main features as in fig. 2.

Fig. 4.—Drawing [× 400] from vertical section through embryonic pole of a more advanced egg (Specimen O). This egg measured about 4·75 millims. in diameter. The vitelline membrane is seen to be intimately lined by the attenuated ectodermal membrane, which is itself in tolerably intimate contact with the yolk-mass. Over the central portion of the white-yolk-bed are seen three plumper-looking deeply placed cells.

Fig. 5.—Composite drawing [× 400] from two sections passing vertically through the embryonic region of the blastoderm and the underlying yolk of a further advanced egg (Specimen "alpha"), measuring 6·5 × 6 millims. in diameter. The yolk-entoderm is already differentiated in contact with the as yet coherent yolk-mass. An accumulation of cells, seemingly derived by proliferation from the ectoderm, has taken place in a position corresponding to the centre of the white-yolk-bed. This is, presumably, an early stage in the formation of the "primitive knot."

Fig. 6.—Stereophotograph [× 1·6] of hemisected uterus (left) and egg *in situ*. (Specimen Y.) The hemispheroidal segment of the egg shows the shell with the blastodermic membrane detached from its cut margin. Within the concavity of the blastoderm (and nearer to the lowest portion of its cut margin) there is visible a small whitish dot which is the now fully developed "primitive" or "archenteric knot" in the "gastrular stage."

Fig. 7.—Photomicrograph [× 350] from section through the bilaminar wall of the blastodermic vesicle in the "gastrular" stage, away from the region of embryonic differentiation.

Fig. 8.—Photomicrograph [× 182] of inner surface of a portion of the bilaminar wall of the blastodermic vesicle in the "neurular" stage, showing the form of the yolk-laden entoderm cells of the "area opaca." Over a limited area the entoderm cells have been removed, and there the much smaller and clearer ectoderm cells are visible. The appearance here displayed is characteristic also of the preceding "gastrular" and "postgastrular" stages.

Fig. 9.—Photomicrograph [× 220] of sagittal section through the archenteric knot in the gastrular stage. (Specimen DD.)

Fig. 10.—Photomicrograph at same magnification of another sagittal section of the same knot. (See text-description.)

Fig. 11.—Photomicrograph [× 225] of transverse section across the "primitive" or "archenteric knot" in another specimen of the "gastrular" stage (Specimen Y). [*Cf.* text-fig. 4 and description in text.]

Figs. 12–20.—Series of photomicrographs [× 350] of sections transversely across the axial region (except fig. 19) of the "primitive-streak area" of Specimen Q. The planes of section are indicated by the numbered cross-lines in text-fig. 7, in the body of the paper. [The plane of fig. 19 is not so indicated since it lies altogether laterally to and outside of the axial region. It is from the more lateral portion of the section whose axial portion is figured in fig. 18.] More detailed description in the text, pp. 56–59.

Fig. 21.—Drawing [× 310] from transverse section across the primitive streak in the "gastrular" stage. (Specimen DD.)

Fig. 22A and B.—Stereophotograph [× 4] of deep or entodermal aspect of the embryonic hemisphere of Specimen P. The blastoderm is in intimate apposition with the deep aspect of the vitelline membrane and shell. The primitive streak, HENSEN'S knot and the posterior portion of the "head-process" or archenteric axis are plainly seen. On either side of the anterior portion of the primitive streak and of the archenteric axis, the differentiation of a "Stammzone" may be discerned through its slightly darker shading, as well as very faint indications of a possible metameric segmentation of this zone.

Fig. 23.—Photomicrograph [× 10] of embryonic region of same blastoderm as shown in fig. 22. The cephalic region of the embryonic area was obscured owing to the extreme curvature of the blastoderm and has been cut away. Compare with the reconstruction-text-fig. 8, and with the text-description, pp. 61–67.

Fig. 24.—Photomicrograph [× 350] from section through the bilaminar extra-embryonic vesicle-wall in Specimen P.

Fig. 25.—Photomicrograph [× 9] of embryonic region of the blastoderm of Specimen E, the shell and vitelline membrane having in this case been removed except over one corner of the preparation. The anterior portion of the embryonic area is lacking and some creasing of the blastodermic membrane has resulted from its flattening-out. The photograph was taken in cedar oil, by transmitted light. The outer or ectodermal surface is uppermost.

Fig. 26.—Drawing [× 350] from transverse section across the primitive streak of Specimen P of the "postgastrular" stage. The reference-letters "*pr. s.*" point to the axial strip of irregular cells which constitutes the primitive streak as seen in figs. 22 and 23. Along this axial strip, here shown in transverse section, the distinction of mesodermal from ectodermal cells is in abeyance.

Fig. 27.—Drawing [× 350] from transverse section across the posterior, more expanded extremity of the primitive streak of Specimen P, showing the thickening of the "Caudalknoten." In this plane the yolk-entoderm exhibits the characteristic features of entoderm of the area opaca.

Fig. 28.—Drawing [× 350] from section made at right angles to the embryonic axis, but behind the primitive streak and the crescentic mesodermal thickening connected with it (*cf.* text-fig. 8). In this backward prolongation of the axial region the ectoderm is still slightly thickened, but wholly free from the underlying thin mesodermal sheet, which here covers the thick entoderm of the area opaca.

Figs. 29–33.—Photomicrographs [× 350] of five consecutive sections passing transversely across the posterior extremity of the archenteric (HENSEN'S) knot, behind the blastopore. (Fig. 29 passes actually through the blastoporic aperture.) The reference-letters "*pl. bl.*" in the series 30–33 indicate the gradually diminishing hinder extremity of the knot, which comes to an end immediately behind the plane of fig. 33. The series well illustrates the relative independence of the knot from the surrounding ectoderm. Compare with text-fig. 10.

Fig. 34.—Photomicrograph [× 350] of transverse section across posterior part of archenteric knot, passing through the blastopore, of Specimen E.

Fig. 35.—Photomicrograph [× 350] of transverse section across posterior part of archenteric (HENSEN'S) knot, just behind the blastopore in Specimen H, belonging to the "neurular" stage.

Figs. 36–40.—Photomicrographs [× 350] of transverse sections (viz., the 1st, 3rd, 4th, 7th, and 9th) in front of the plane of fig. 29. Fig. 36 lies in the plane of the transversely extended blastoporic aperture, at least on the left side. The remaining figures illustrate the massive knot-like thickening of the archenteron, containing vestiges of its lumen. They also illustrate the progressive differentiation of the ectoderm overlying the thick archenteron. (Specimen P.)

Figs. 41–43.—Photomicrographs [× 350] of three sections across the archenteric (HENSEN'S) knot in Specimen E Fig. 41 passes very shortly in front of the blastoporic aperture. Figs. 42 and 43 are respectively 0·03 and 0·05 millim. in front of the plane of fig. 41. These figures also illustrate the differentiation and establishment of the ectoderm from the superficial portion of the archenteric knot-tissue.

Figs. 44–48.—Photomicrographs [× 350] from transverse sections across the archenteron of Specimen P in the region of occurrence of ventral archenteric defect (*cf.* text-fig. 10). Fig. 44 shows the archenteric parietes complete, and underlain by a nearly complete layer of yolk-entoderm. Figs. 45–48 illustrate the three consecutive sections next but one in front of the plane of fig. 44. They show the progressive disappearance of the archenteric floor and the consequent denudation of the archenteric roof to form the "archenteric plate."

Fig. 49.—Drawing (at a reduction of one-third) of the ventral aspect of a wax-plate

model [× 400] of the archenteric knot and the adjacent part of the archenteric plate in Specimen P. In the construction of the model the entoderm was left out altogether. The figure should be compared with the mesial-plane reconstruction reproduced in text-fig. 10.

The knob-like projections on the denuded surface of the archenteric plate are due to the persistence of cellular masses as illustrated in figs. 47 and 50.

Fig. 50.—Photomicrograph [× 350] of transverse section across archenteric plate (Specimen P), some distance in front of plane of archenteric defect. A knob-like projection of cellular tissue is seen adherent to the free surface of the archenteric plate. This tissue is a remnant of the otherwise disintegrated ventral portion of the archenteric parietes and a yolk-entoderm cell is still seen adhering to its ventral aspect.

Fig. 51.—Photomicrograph [× 350] of transverse section across archenteric plate in Specimen P, at a distance of 0·9 millim. in front of the blastopore. At this distance forward the archenteric plate has entirely lost its rugged and irregular character and its thickness has become reduced to one layer of cells.

Fig. 52.—Photomicrograph [× 350] of transverse section across intact archenteron of Specimen PP, 0·02 millim. behind the plane of ventral archenteric defect and 0·18 millim. in front of the blastopore. The partial differentiation of the tissue of the archenteric parietes into cell-masses is illustrated.

Fig. 53.—Photomicrograph [× 350] of transverse section next behind the ventral archenteric defect in Specimen PP, showing somewhat more pronounced differentiation of the "protosomitic" cell-masses.

Fig. 54.—Photomicrograph [× 350] of section in front of last, passing through the plane at which disintegration of the ventral archenteric wall sets in. This plane is 0·2 millim. in front of the blastoporic aperture. Ventrally, cellular debris, in the form of a few nuclei and reticular material, represents the disintegrated archenteric floor, together with the yolk-entoderm clothing the ventral aspect of the latter.

Fig. 55.—Photomicrograph [× 350] of transverse section across the archenteric plate in Specimen PP, 0·12 millim. in front of the plane of fig. 54.

Fig. 56.—Photomicrograph [× 350] of transverse section across archenteric plate in Specimen PP, 0·03 millim. in front of the plane of fig. 55, showing differentiation of medial and lateral regions of the archenteric plate.

Fig. 57.—Photomicrograph [× 350] of third section in front of plane of fig. 56, showing a well-marked layer of reticulum adhering to the ventral aspect of the plate, and representing the remains of archenteric cellular material which has undergone disintegration.

Figs. 58–67.—Series of photomicrographs [× 350] of transverse sections across

archenteric knot and posterior part of archenteric plate in Specimen E. This series of figs. illustrates the organisation of the archenteric tissue into the protosomitic cell-masses, and the transition of these anteriorly —whilst suffering ventral denudation—into the archenteric axial plate.

The plane of fig. 58 is 0·06 millim. in front of that of fig. 43; fig. 59 0·06 millim. in front of fig. 58; fig. 60 0·06 millim. in front of fig. 59; fig. 61 0·07 millim. in front of fig. 60; fig. 62 represents the next section in front of that shown in fig. 61; fig. 63 0·018 millim. in front of fig 62; fig. 64 0·012 millim. in front of fig. 63; fig. 65 0·018 millim. in front of fig. 64; fig. 66 is the succeeding section to that in fig. 65; fig. 67 illustrates a plane 0·078 millim. in front of that of fig. 66.

Fig. 68.—Photomicrograph [× 220] of longitudinal and approximately mesial section through the region embracing the anterior extremity of the primitive streak, the archenteric or HENSEN's knot, and the posterior portion of the archenteric plate in an embryo of the "postgastrular" stage of *Perameles obesula*.

"*n.*" = slight superficial notch marking anterior boundary of the primitive streak tissue. "*bl. dep.*" = the surface depression representing the aperture of the blastopore. "*s. d.*" = a shallow surface depression in front of the prominence of the undifferentiated cellular tissue of the knot. The depression corresponds with the place at which the differentiated ectoderm reaches the middle line (*cf.* text-fig. 12). The indifferent knot-tissue behind the depression is continued back to form the anterior or dorsal lip of the blastopore.

Anteriorly, at "*op.*," the floor of the forwardly prolonged archenteron disappears abruptly, exposing in front the archenteric roof or "plate." The intact portion of the archenteron, behind the plane marked by "*op.*," shows no actual lumen. Immediately behind "*op.*," however, a virtual lumen may be detected and followed backwards for a short distance as a fine line.

[*Note to description of fig.* 68.—In preparing this print for reproduction it was inadvertently cut across just about the plane of quasi-continuity between the anterior end of the primitive knot and the cellular tissue of the posterior portion of the archenteric knot. This accidental cut passes through the notch referred above under the reference-letter "*n.*" The cut edges were replaced in accurate apposition and the figure has in no other way been interfered with. The negative being in Australia, it has not been possible to replace the print by another perfect copy.]

Fig. 69.—Photomicrograph [× 220] of longitudinal, approximately mesial section through anterior portion of same embryo as that of fig. 68, showing anterior portion of archenteric plate with its "protochordal-plate" segment.

Fig. 70.—Photomicrograph [× 220] from section in plane adjoining that of fig. 68. Here the posterior limitation of the tissue of the archenteric knot from that of the primitive streak is even more obvious than in fig. 68.

Fig. 71.—Photomicrograph [× 220] from transverse section across the anterior thickened terminal segment of the archenteric plate ("protochordal-plate" segment) in specimen of *Perameles obesula* of same stage as that of figs. 68–70.

Fig. 72.—Photomicrograph [× 10·5] of convex ectodermal aspect of the blastodermic vesicle of *Perameles obesula*. The stage is the same as that from which the sectional figs. 68–71 are taken and corresponds to that which has been designated in the case of Ornithorhynchus as the "postgastrular." For general description of this figure reference may be made to pp. 141 and 142 of the paper. (This and the six following figures are on Plate 1.)

Fig. 73.—Photomicrograph [× 6·4] of ventral surface of embryonic area of blastoderm of Ornithorhynchus, "neurular" stage (Specimen ZZ), taken in alcohol by reflected light. Description in text, pp. 117–121.

Fig. 74.—Photomicrograph [× 6·4] of ventral aspect of embryonic area of blastoderm of Ornithorhynchus, "neurular" stage (Specimen Z), taken by transmitted light after lightly staining and clearing in cedar oil. Description in text, pp. 118–121.

Fig. 75.—Photomicrograph [× about 6 diameters] of ventral aspect of embryonic area from late "neurular" stage of Ornithorhynchus (Specimen M). Description in text, pp. 121–129.

Figs. 76–77.—Photomicrographs [× 8] of dorsal aspect (*i.e.*, ectodermal) of the anterior and posterior portions of the embryonic area from late "neurular" stage of Ornithorhynchus (Specimen H). Description in text, pp. 121–129. The numbered lines in fig. 76 indicate the planes of corresponding sectional photomicrographs.

Fig. 78.—Photomicrograph [× 10·5] of dorsal (ectodermal) aspect of embryonic area of blastodermic vesicle of *Dasyurus viverrinus*. Description in text, pp. 140–144.

Figs. 79–80.—Photomicrographs [× 350] of two sections, both passing transversely across the somite which lies seventh from the posterior end of embryo in Specimen H (*cf.* fig. 77).

Fig. 81.—Photomicrograph [× 78] of transverse section across anterior margin of second prefacial neuromere in cephalic medullary plate of Specimen H, showing general sectional characters of trigeminal lobe. Compare with corresponding reference-line in fig. 76.

Fig. 82.—Photomicrograph [× 350] of portion of transverse section across middle region of Trigeminus ganglionic plate in Specimen ZZ, showing the free lateral margin, "*m. t. gl.*," of the ganglion-plate.

Figs. 83–96.—Photomicrographs from sections of the cephalic region in the late neurular stage in Ornithorhynchus (Specimens H and M), to illustrate the sectional characters of the neural organisation. In the case of transverse sections from Specimen H, correspondingly numbered reference-lines will be found in fig. 76, to which reference should be made.

Fig. 83.—[× 350] Transverse section, marginal region of medullary plate, etc., in Specimen H, in the plane of the third-prefacial neuromere, with its trigeminal ganglionic proliferation.

Fig. 84.—[× 78] Transverse section, plane of fifth-prefacial neuromere, showing special ganglionic proliferative thickening, "*t. gl.*" Specimen H.

Fig. 85.—[× 350] High-power photograph of part of section shown in fig. 84.

Fig. 86.—[× 175] Transverse section, part of section 0·6 millim. behind anterior margin of medullary plate, and cutting transversely across the optic groove, the lateral head-fold and the anterior prolongation of the Trigeminus ganglionic plate. Specimen H.

Fig. 87.—[× 78] Transverse section, medullary plate and Trigeminus lobe, just behind posterior limit of lateral head-fold in Specimen M. Note the distinction between the neural-crest cells and those of the mesoderm underlying the medullary plate.

Fig. 88.—[× 78] Transverse section, medullary plate in Specimen M, in the plane of the first-prefacial neuromere. Here neural-crest cells are entirely absent.

Fig. 89.—[× 350] Transverse section, region of margin of medullary plate in the plane of the first-prefacial neuromere in Specimen H. Here there is evident a very slight representative of the neural crest.

Fig. 90.—[× 350] Transverse section, region of margin of medullary plate in the plane of the anterior portion of the "facial" neuromere in Specimen H. Here there is present a ganglionic proliferation representing a basal part of the acustico-facial "pedicle."

Fig. 91.—[× 350] Transverse section, region of margin of medullary plate in the plane of the middle portion of the "facial" neuromere in Specimen H. This shows the acustico-facial "pedicle" extending outwards as a ganglionic proliferation from the margin of the neuromere, under cover of the auditory plate. A mesial ganglionic plate-like extension is also seen extending medially in contact with the deep surface of the medullary plate.

Fig. 92.—[× 175] Transverse section across marginal portion of medullary plate, auditory plate, and main mass of acustico-facial ganglion, in the plane of the posterior portion of the "facial" neuromere. The entire extent of the acustico-facial "pedicle" is visible in this section ("*a-f. s.*"), as well as the medial extension, "*m. g.*"

Fig. 93.—[× 175] Longitudinal section of posterior region of cephalic medullary plate in Specimen H, showing various neuromeres in section. These include, from right to left, the first prefacial, the "facial," and the first and second postfacial.

Fig. 94.—[× 175] Transverse section as in fig. 92, but in the plane of the anterior portion of the first-postfacial neuromere in Specimen H. The auditory plate and the acustico-facial ganglion are well seen.

Fig. 95.—[× 175] Transverse section, marginal region of medullary plate, etc., in the plane of the second-postfacial neuromere in Specimen H. The marginal portion of the medullary plate is peculiarly thickened and recurved (*cf.* the neuromere as seen in fig. 76). The hindmost portion of the auditory-plate thickening is still visible, "*aud.*" Beneath this the "vago-glossopharyngeal plate" extends outwards as a definite sheet of cells, "*g. v.*"

Fig. 96.—[× 78] Transverse section, in plane of posterior margin of the third-postfacial neuromere, showing the extension outwards of the "vago-glossopharyngeal plate" or ganglionic lamina as far as the inner limit of the "parietal cavity" or pericardial cœlom, "*a. c.*"

Fig. 97.—[× 78] Transverse section across cephalic region of embryo of *Perameles obesula* of stage figured in fig. 72. Note the zone of extra-medullary neural ectoderm, "*n. ect.*," beyond the limit of the future margin of the medullary plate, which represents the "neural crest."

Fig. 98.—[× 175] Transverse section, cephalic region of embryo of *Dasyurus viverrinus*, of same stage as that figured in fig. 78. The plane of the section lies about 0·15 millim. anterior to the plane of the posterior extremities of the trigeminal plates as seen in fig. 78.

Fig. 99.—[× 175] Another section of same embryo in a plane about 0·25 millim. behind the anterior margin of the "facial" neuromere, showing the outgrowth of the acustico-facial portion of the neural crest.

Fig. 100.—Photomicrograph [× 350] of transverse section passing through the blastoporic aperture of the archenteric knot in Specimen H, about 0·03 millim. in front of the plane of fig. 35.

Fig. 101.—From another section about 0·1 millim. in front of the plane of fig. 100 [× 350].

Fig. 102.—From another section 0·16 millim. in front of plane of fig. 101 [× 350].

Fig. 103.—From another section 1·14 millims. in front of plane of fig. 102 [× 350].

Figs. 104–107.—Series of drawings [× 350] from sections across the chorda-Anlage in Specimen ZZ (early "neurular" stage). Fig. 104 is taken from a plane about 0·4 millim. behind the posterior limit of the "protochordal-plate" expansion. Figs. 105–107 are from three consecutive sections about 1·25 millims. behind the plane of fig. 104. They illustrate the metamorphosis of the narrow "chorda-plate" into the definitive chorda.

(The above four figures are on Plate 3.)

Fig. 108.—Photomicrograph [× 122] of transverse section across the seventh somite from the posterior end of Specimen H (*cf.* fig. 77).

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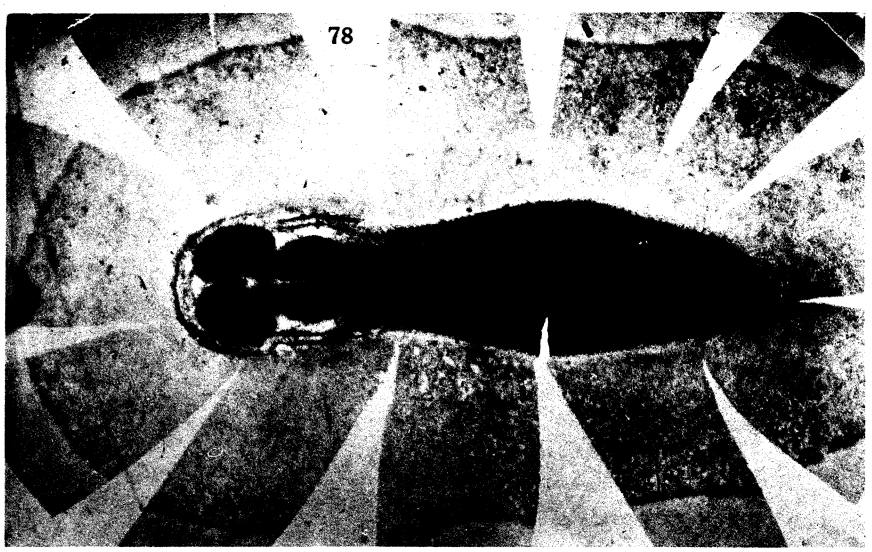

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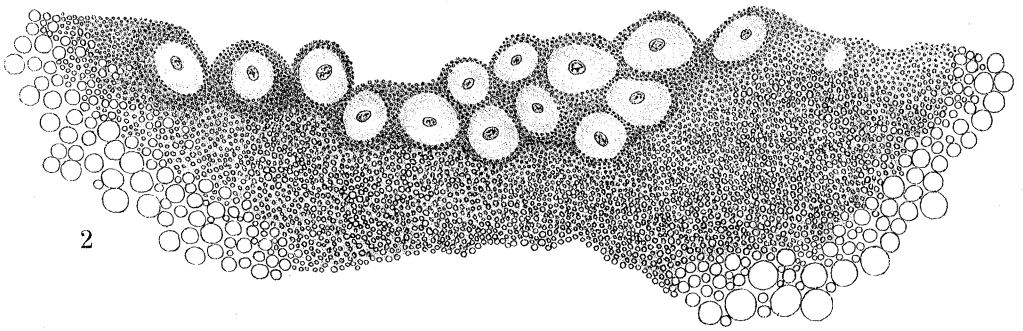

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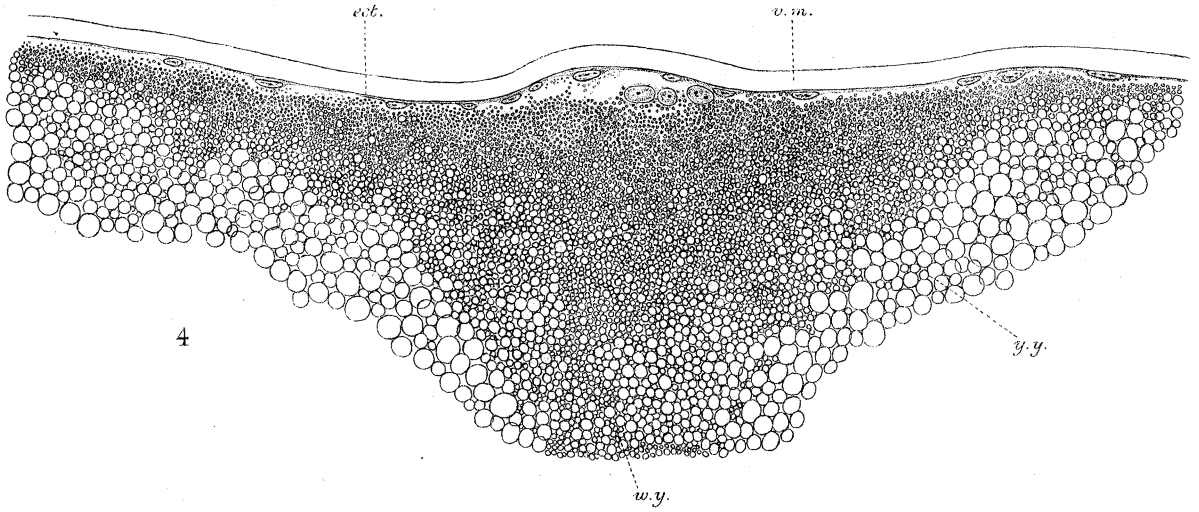

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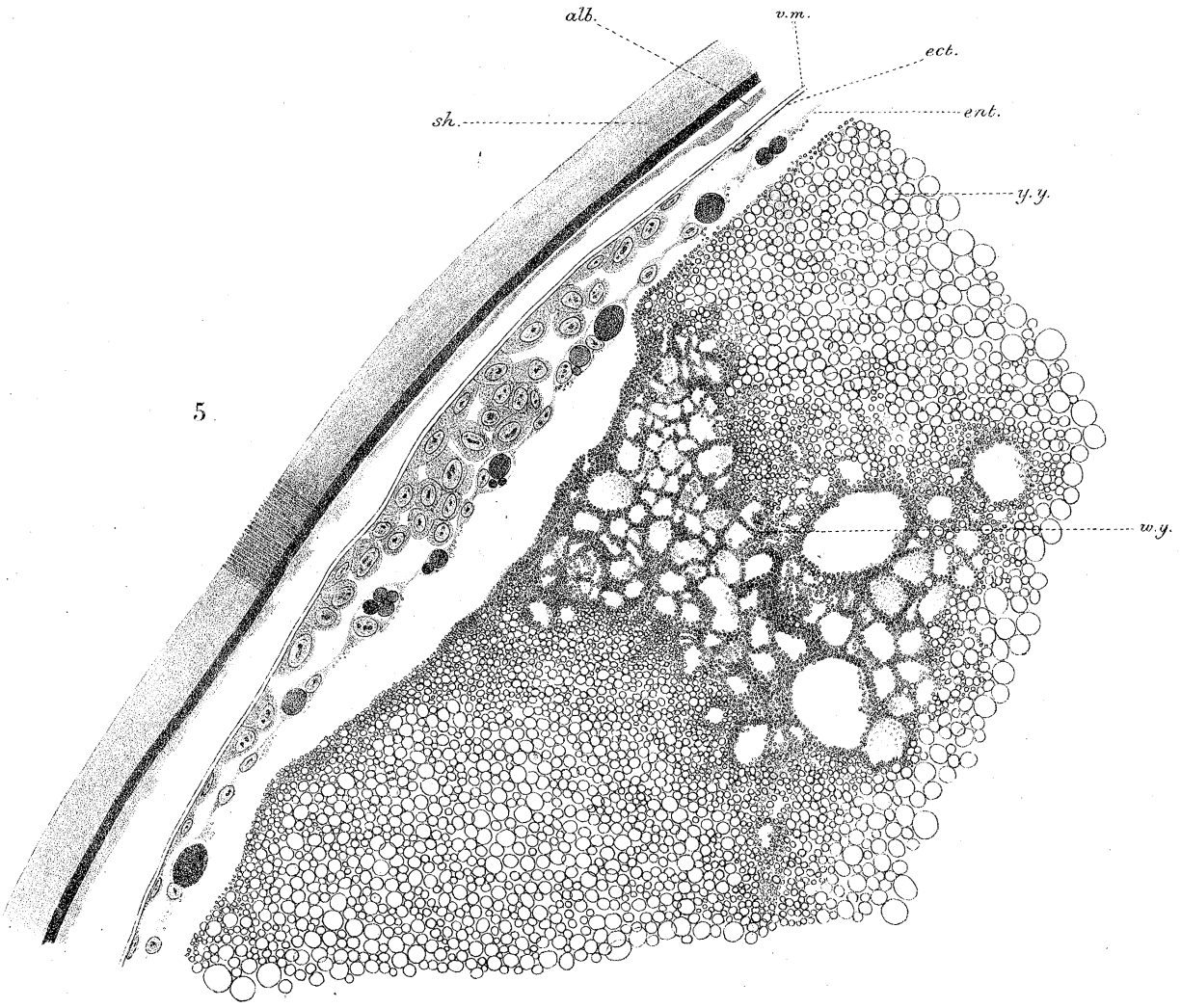

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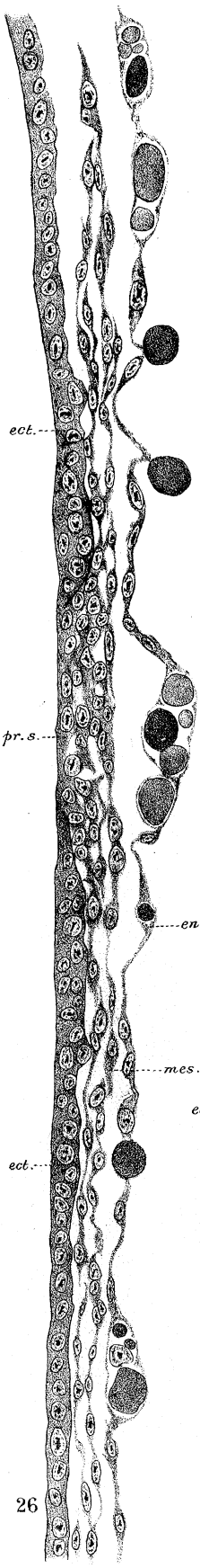

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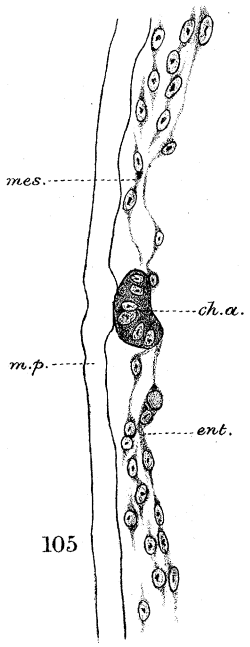

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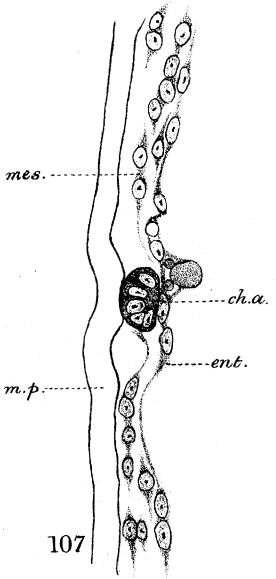

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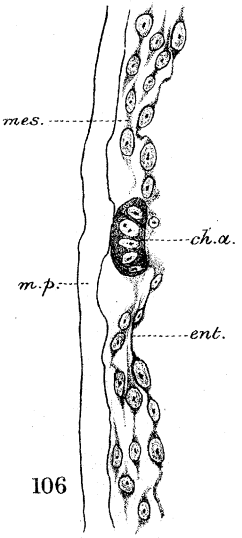

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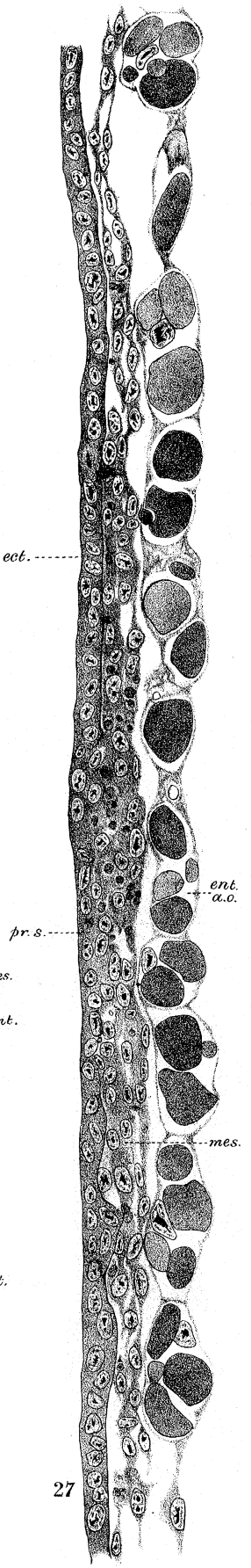

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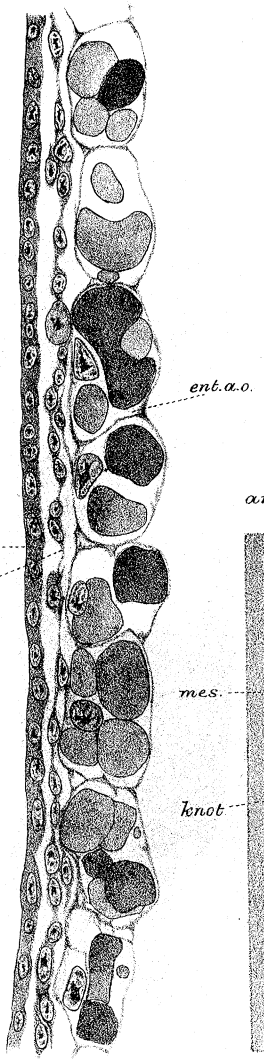

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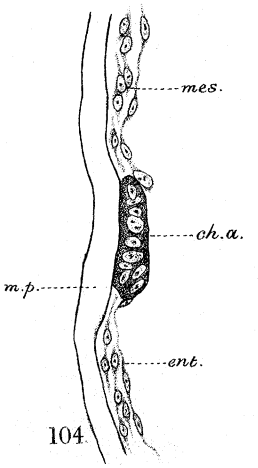

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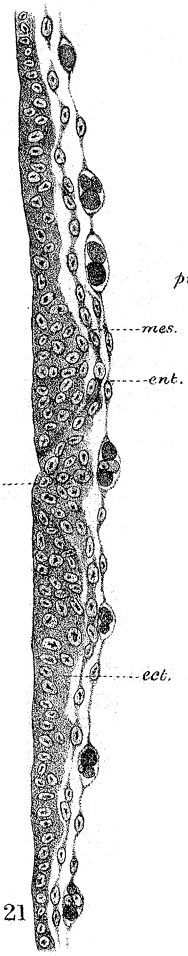

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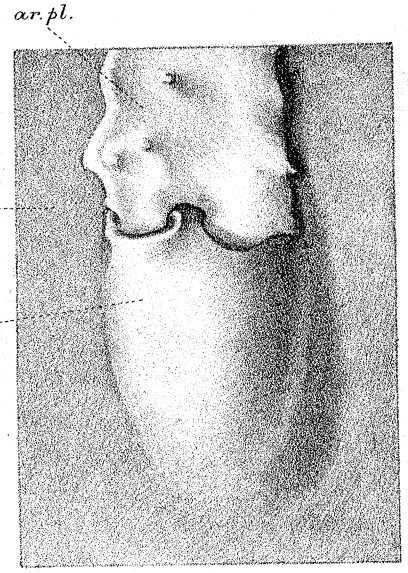

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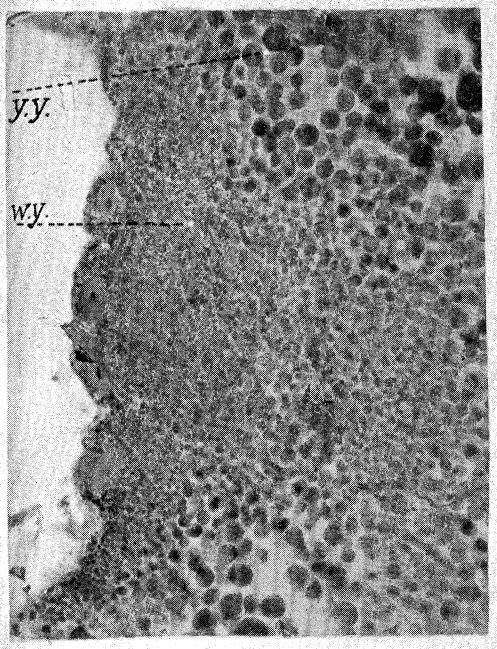
M^cFarlane & Erskine, Lith. Edin^r



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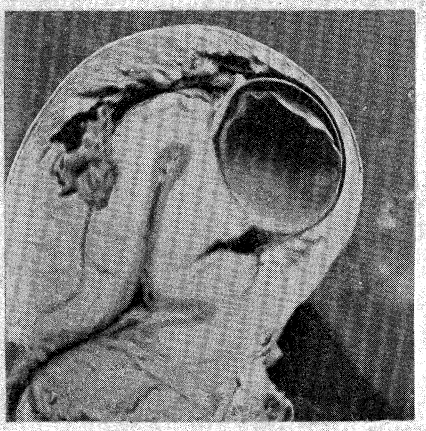

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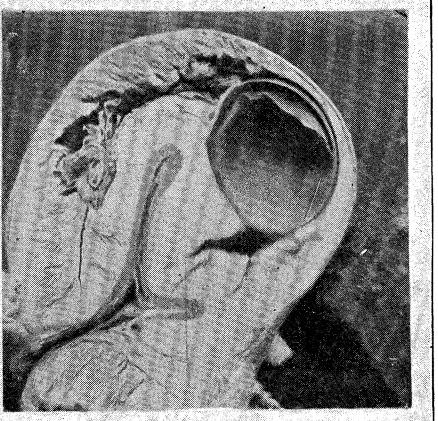

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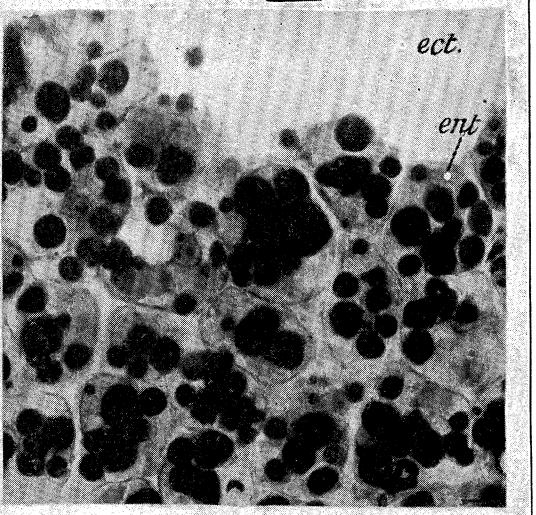

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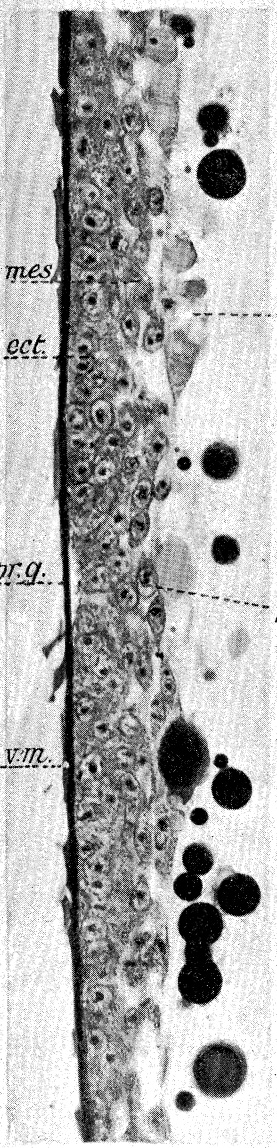

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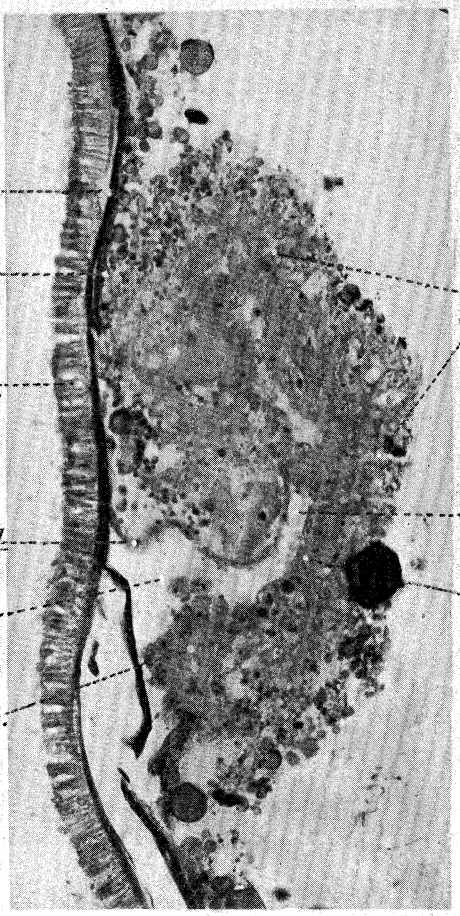

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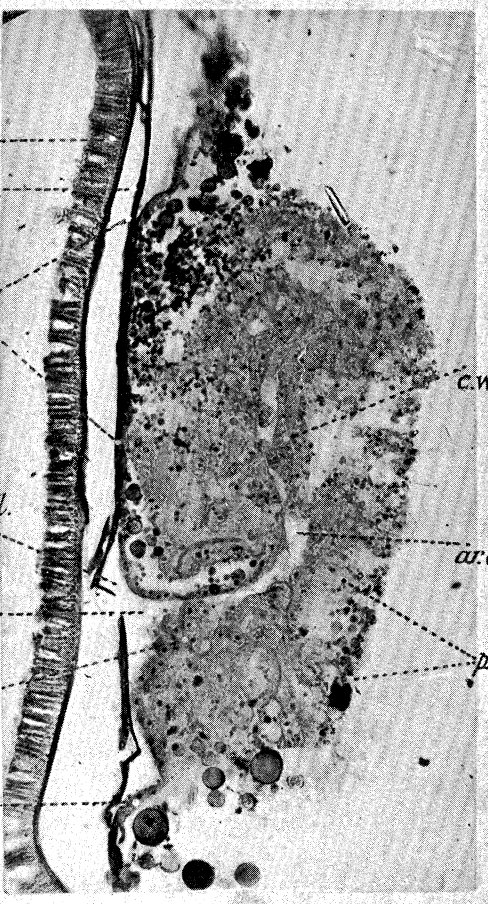

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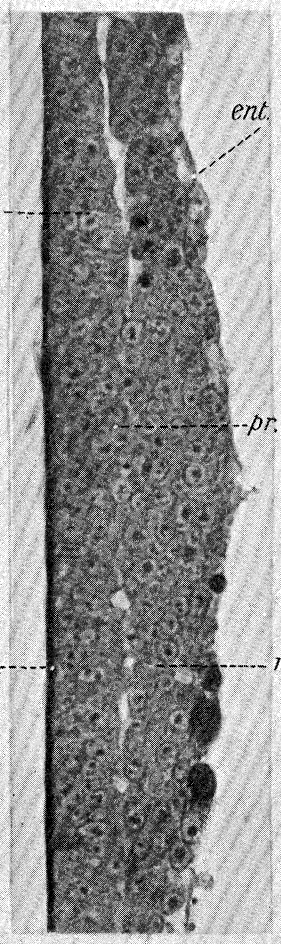

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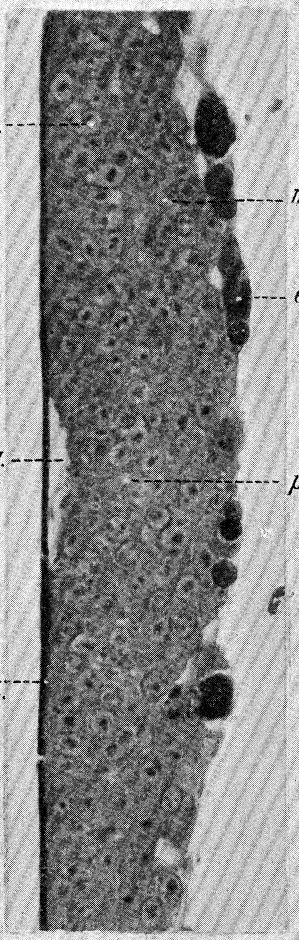

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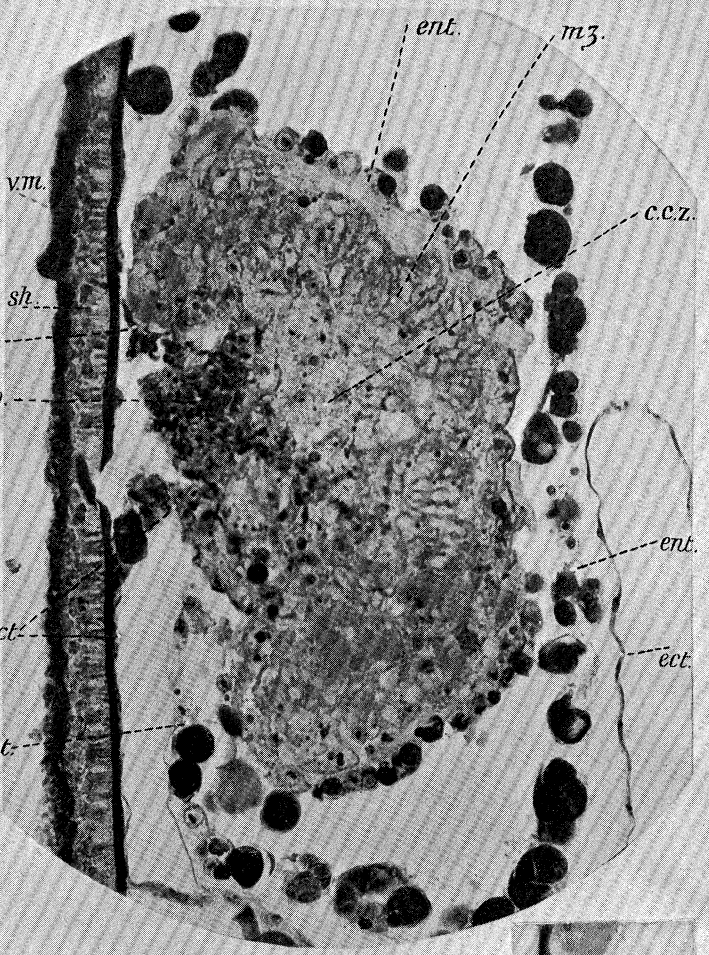

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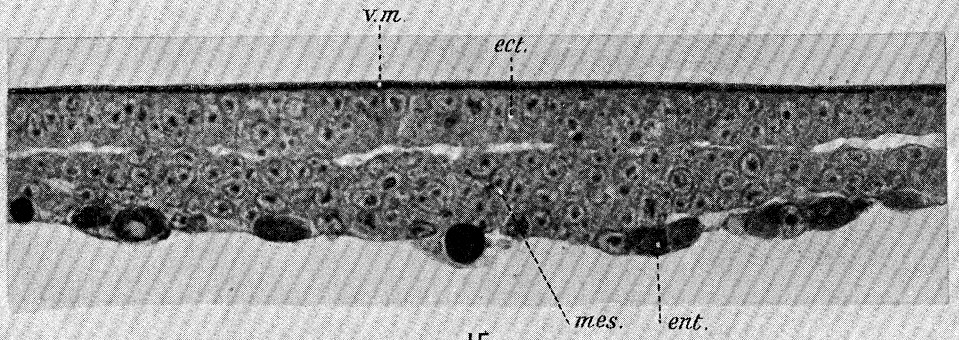

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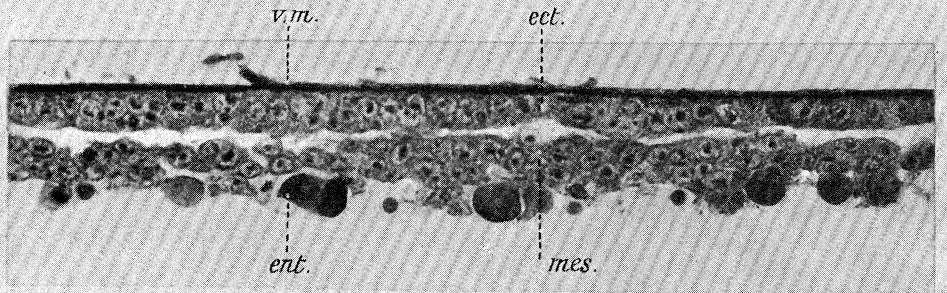

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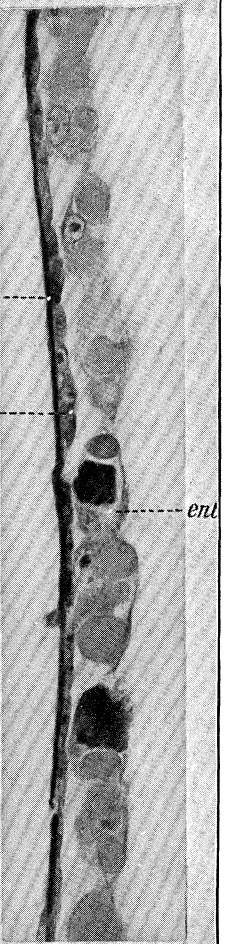

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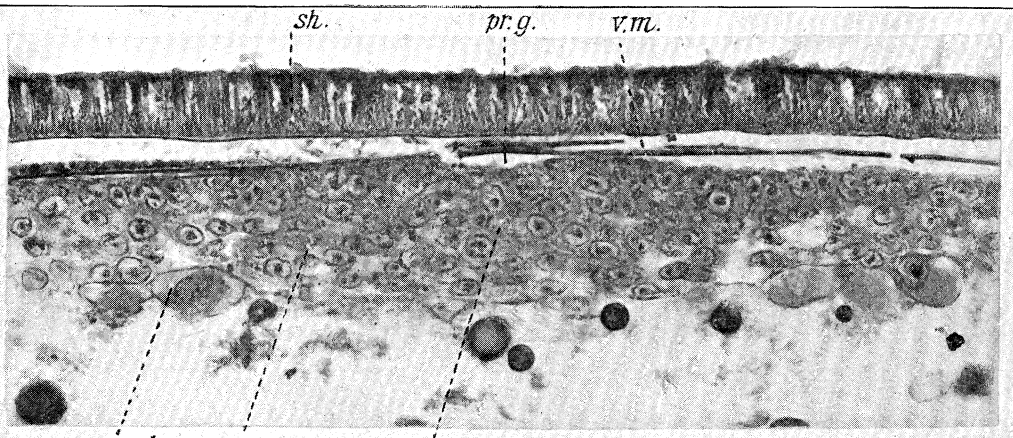

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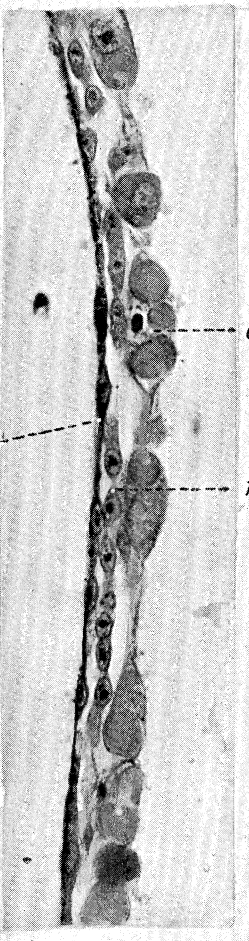

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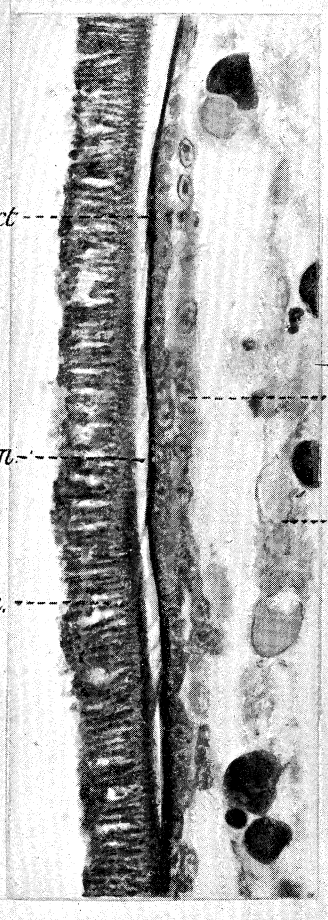

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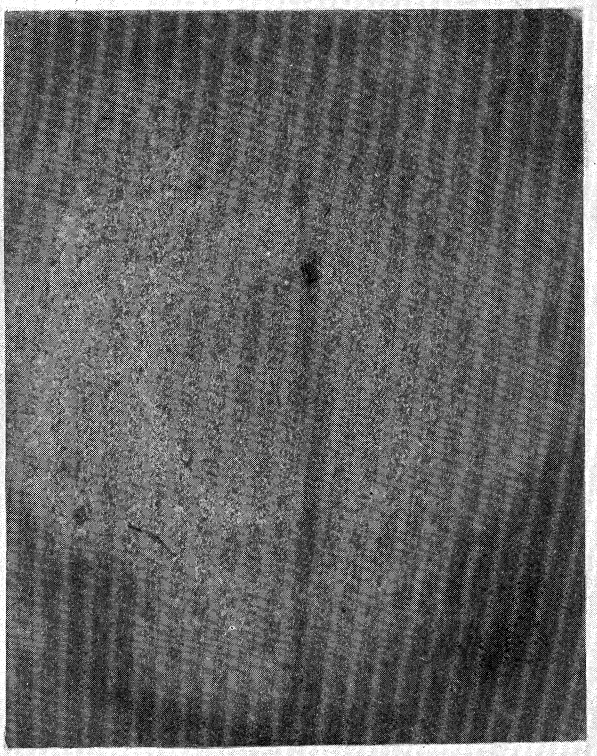

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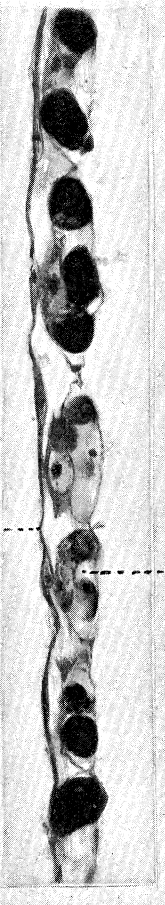

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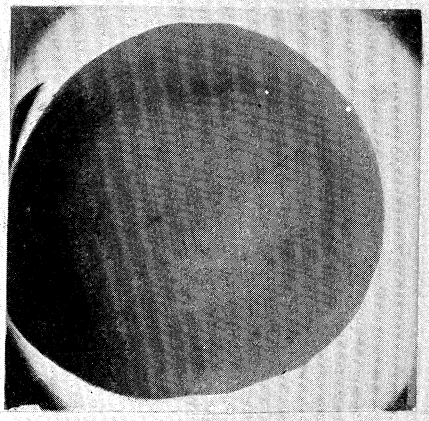

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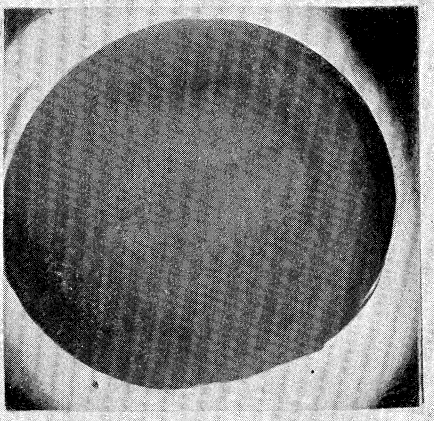

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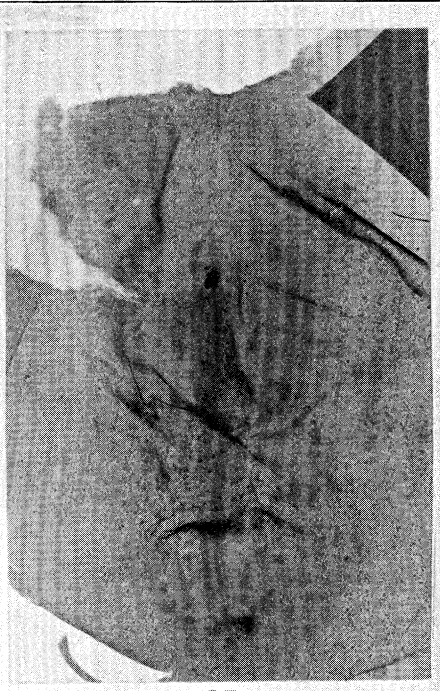

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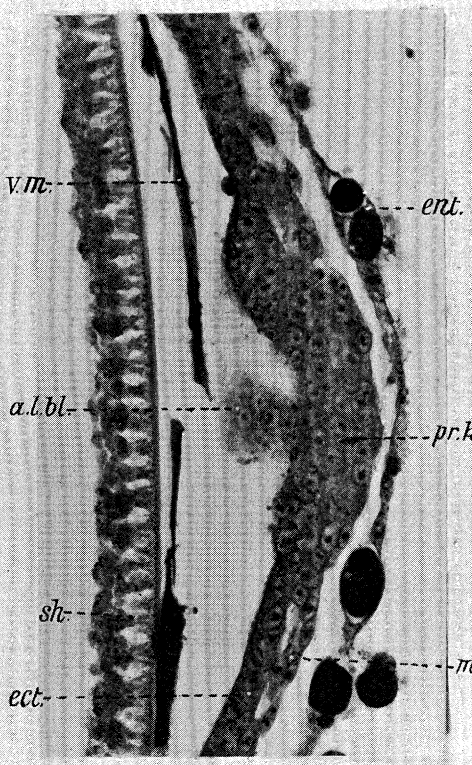

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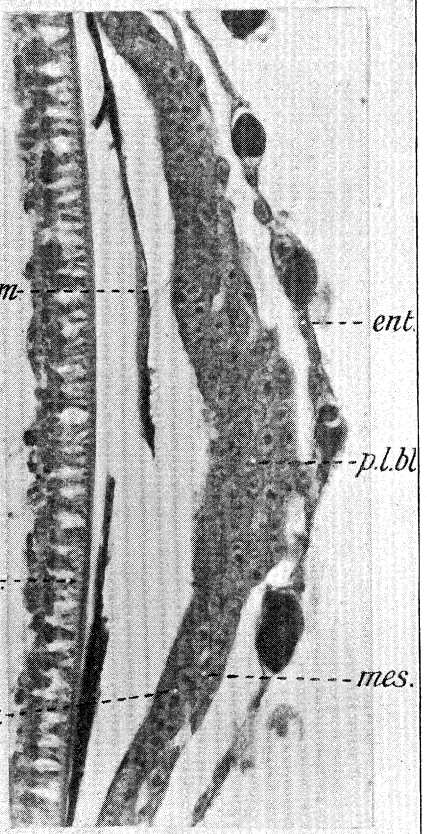

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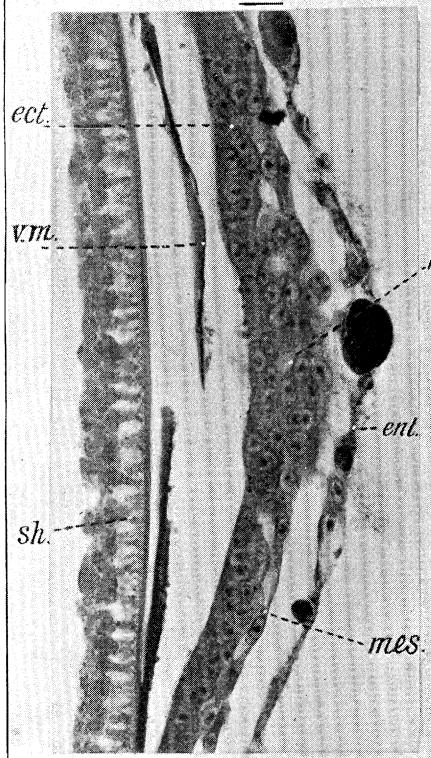

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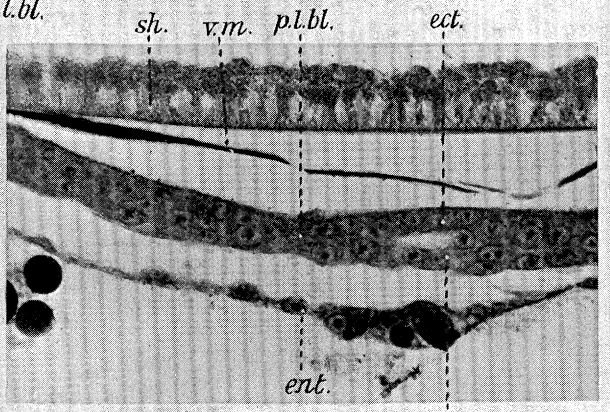

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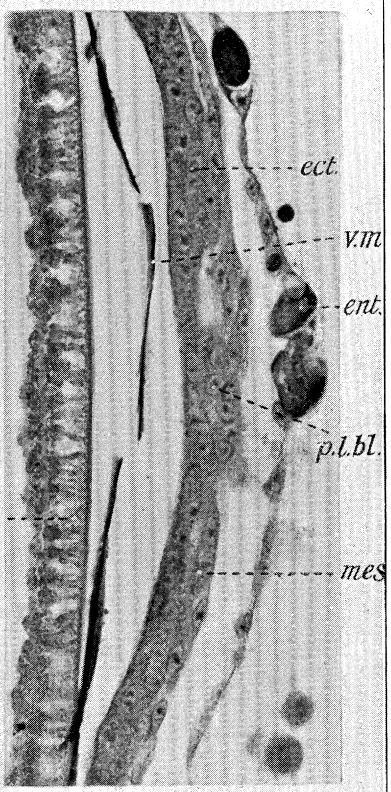

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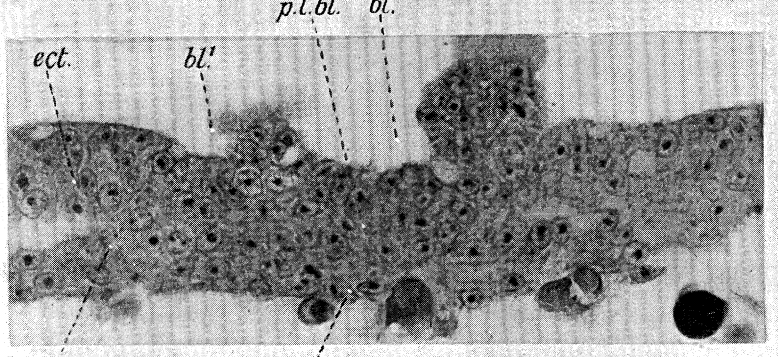

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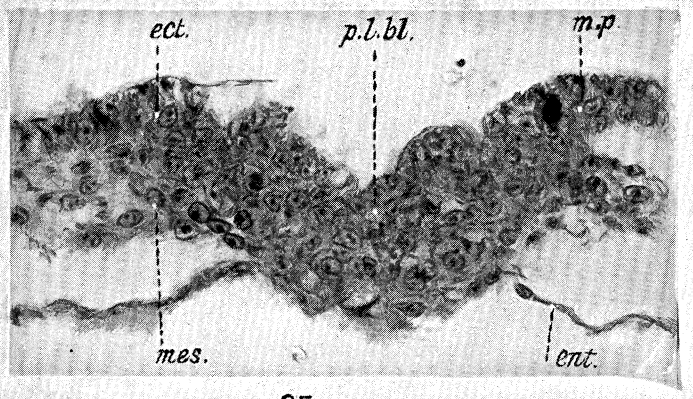

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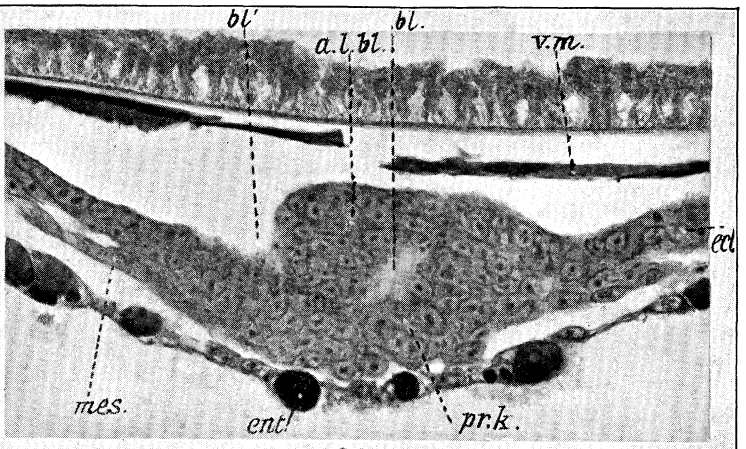

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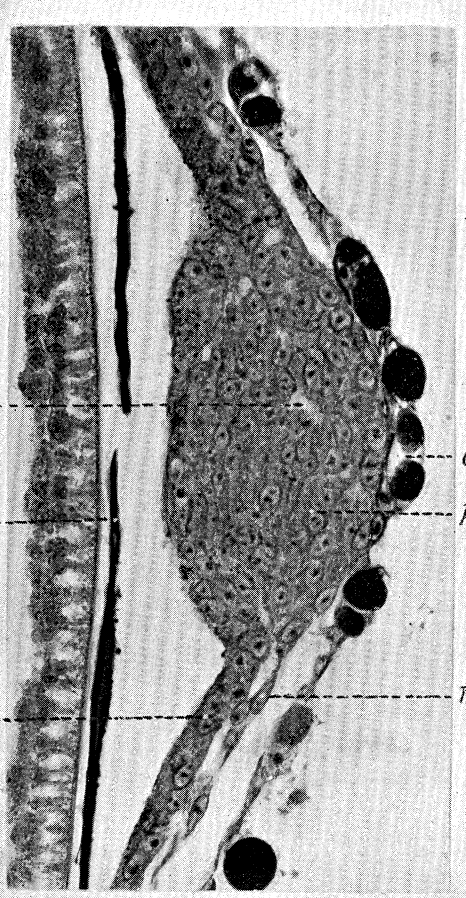

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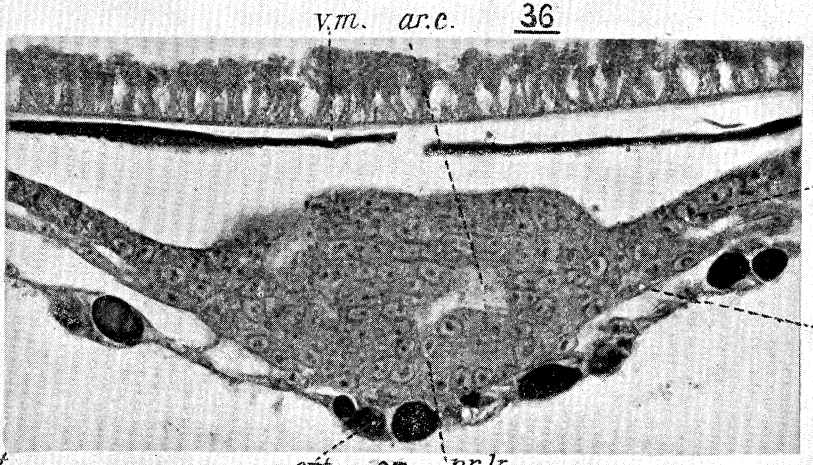

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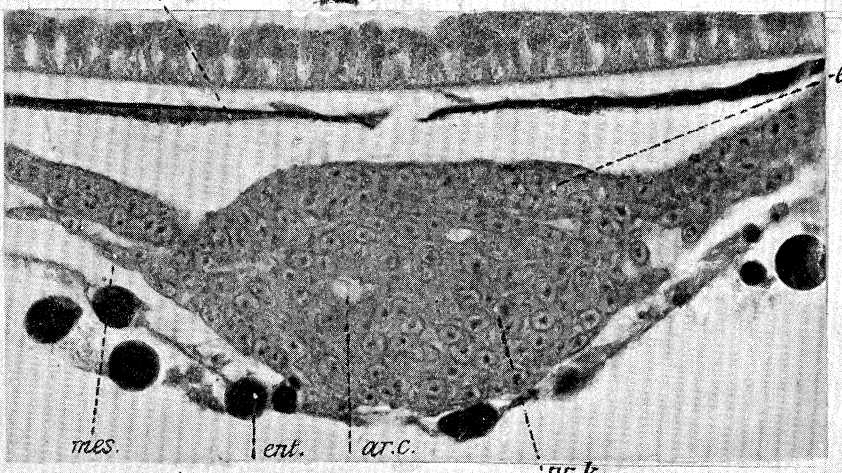

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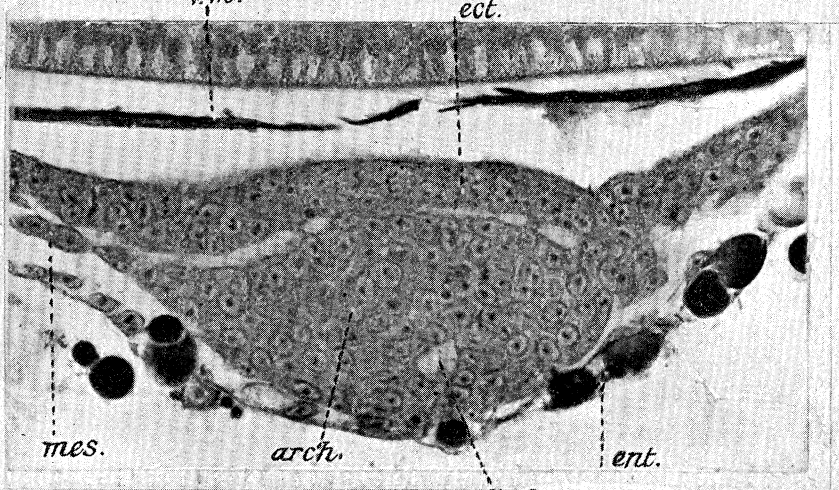

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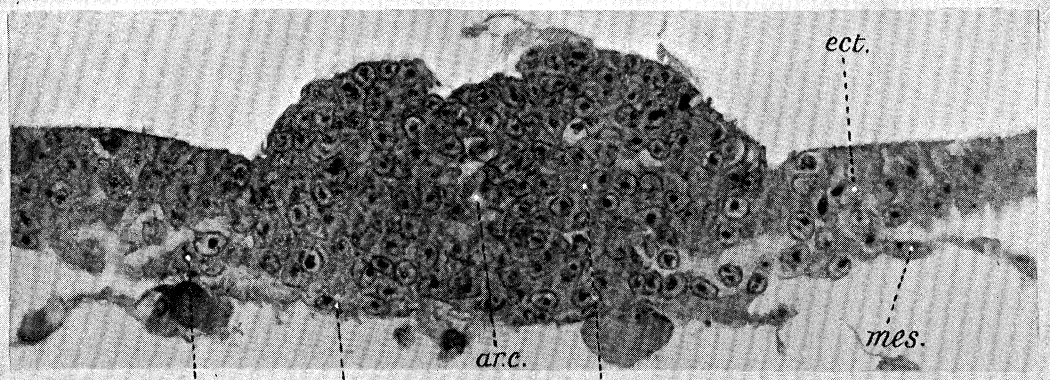

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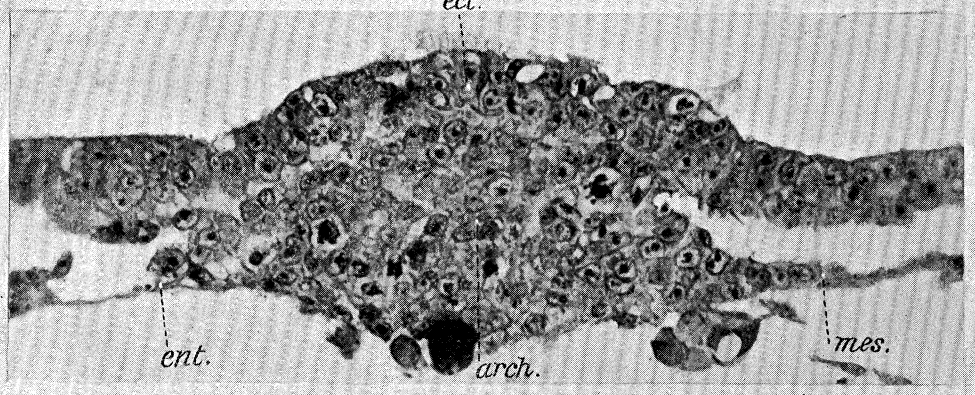

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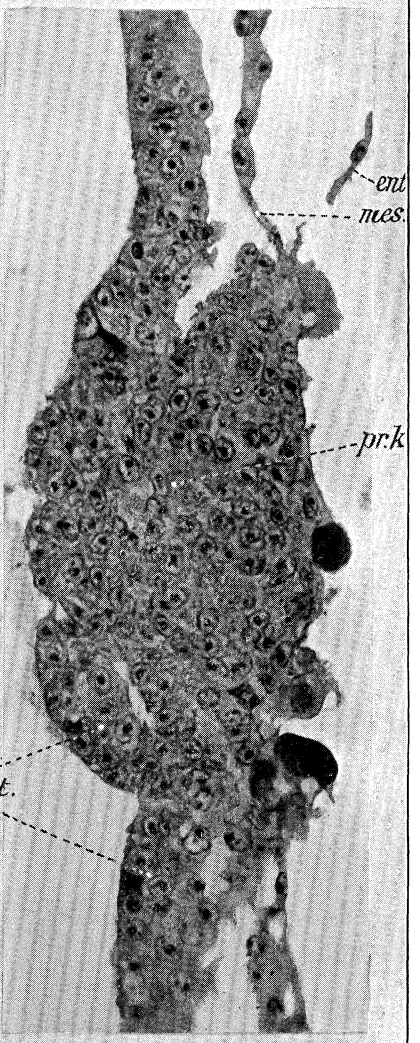

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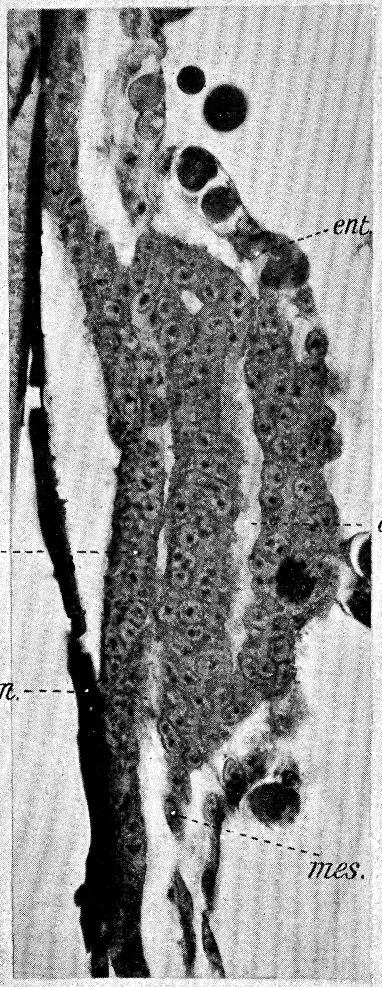

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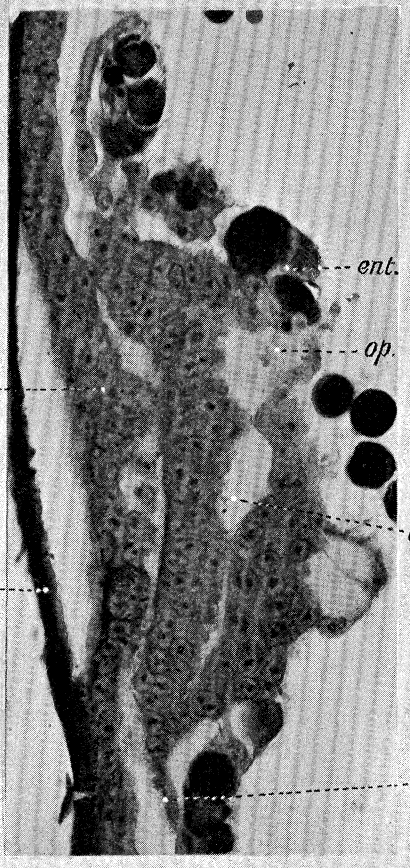

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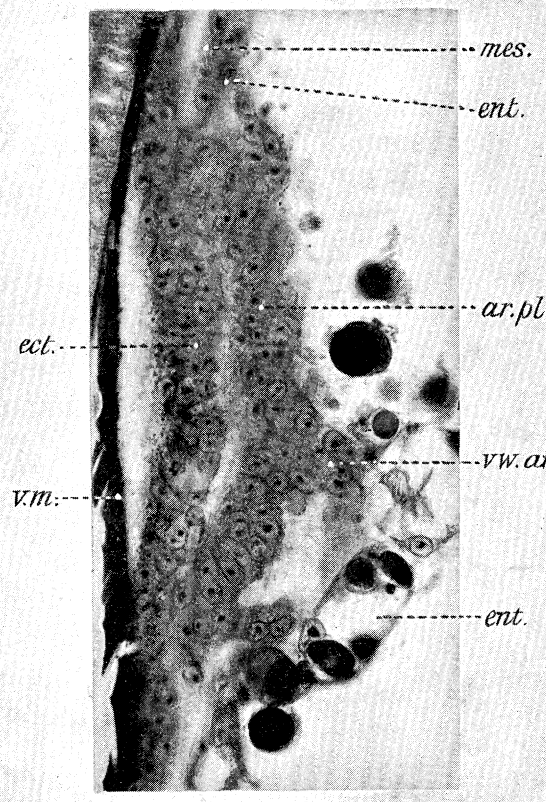

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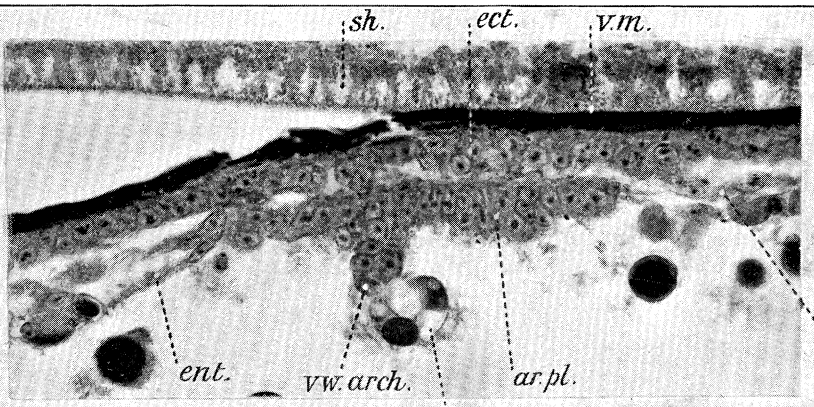

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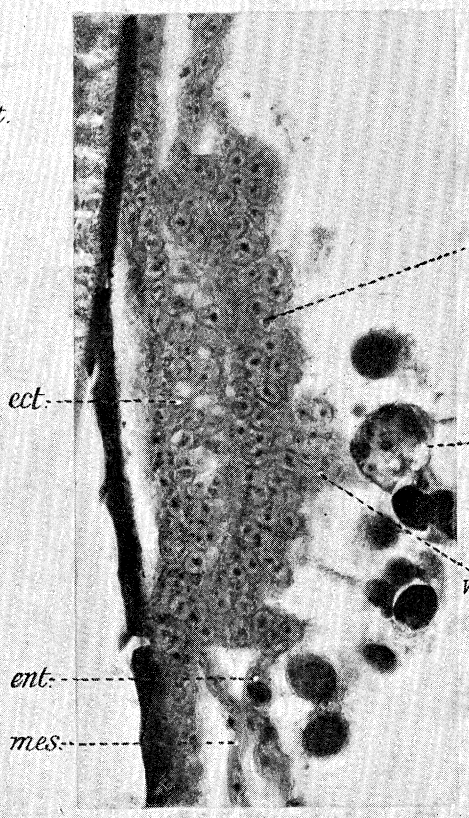

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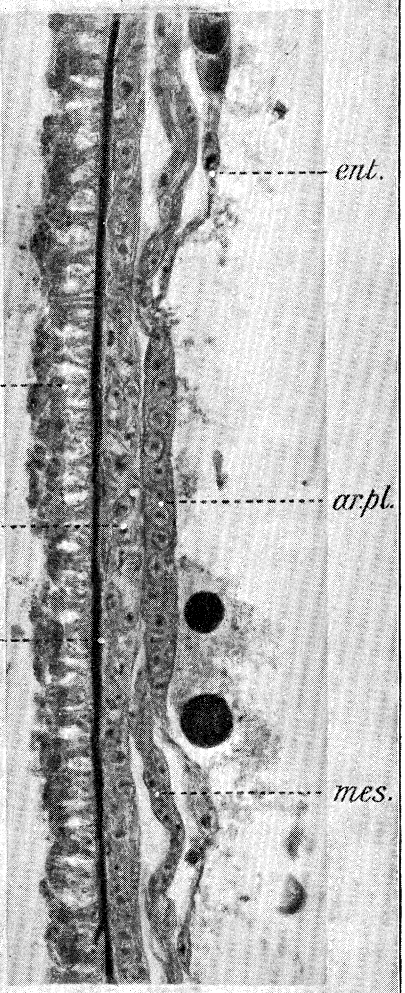

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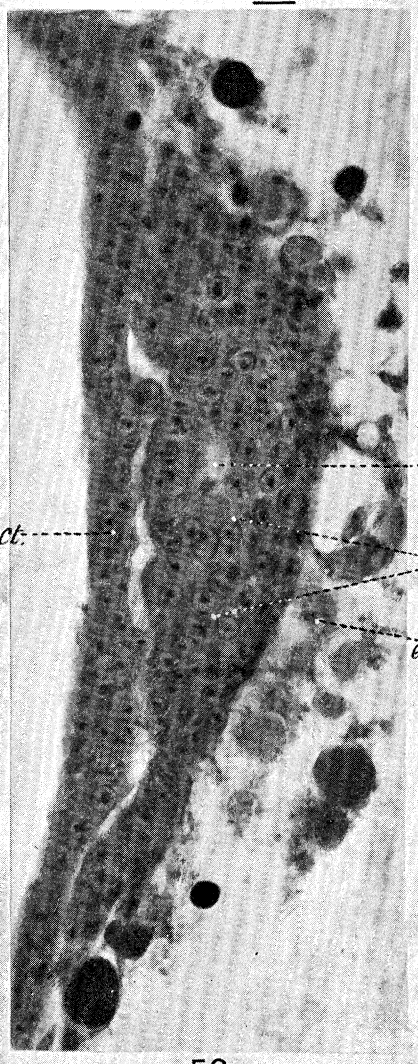

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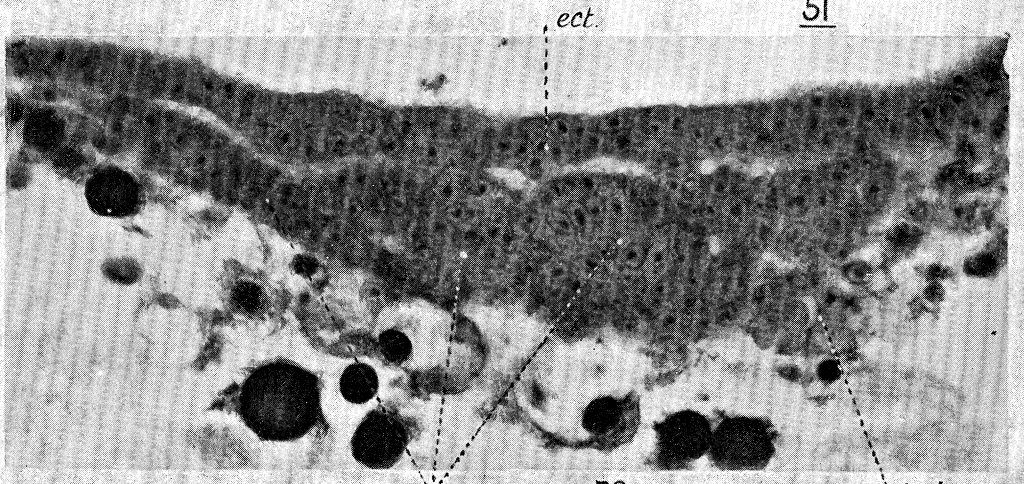

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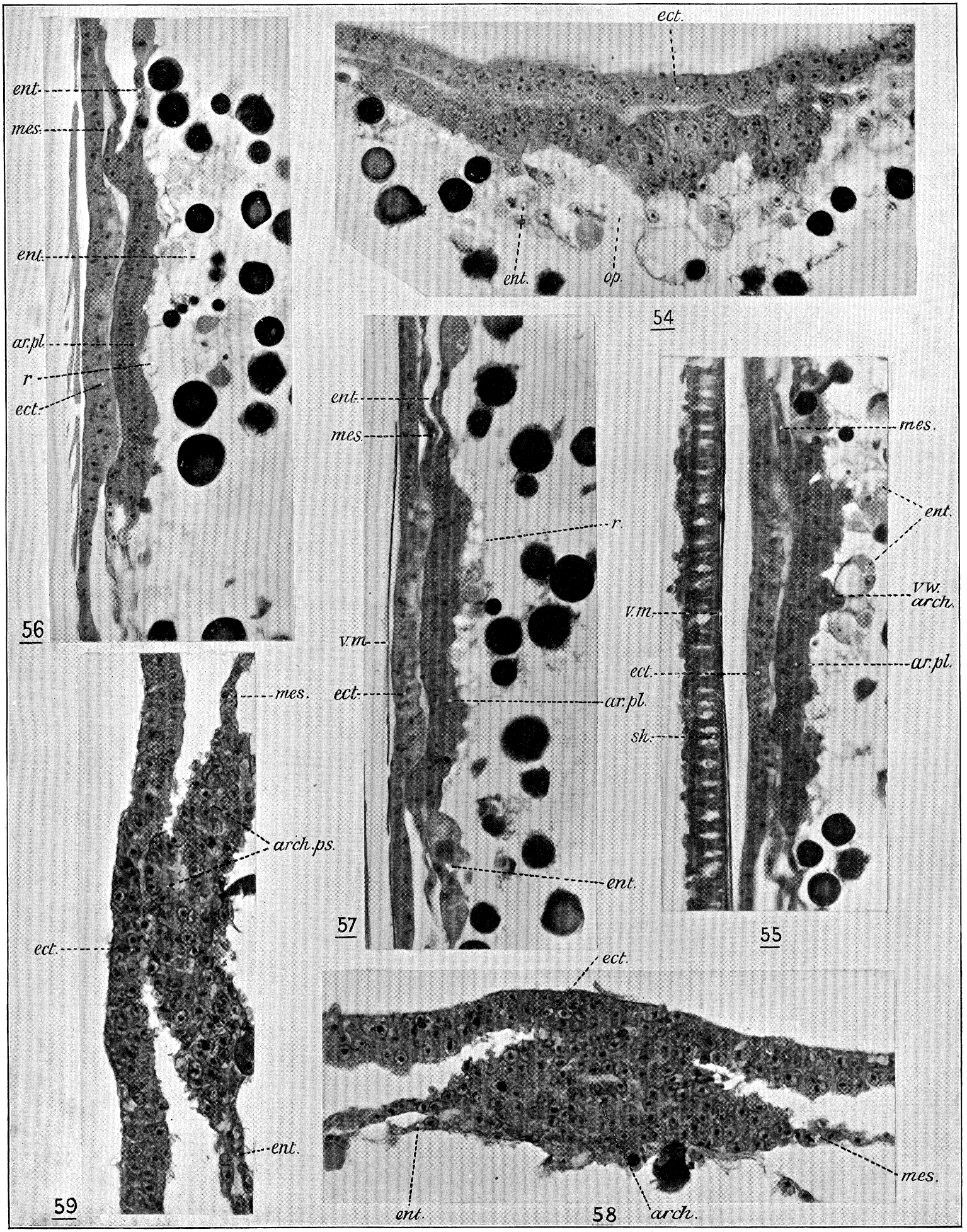
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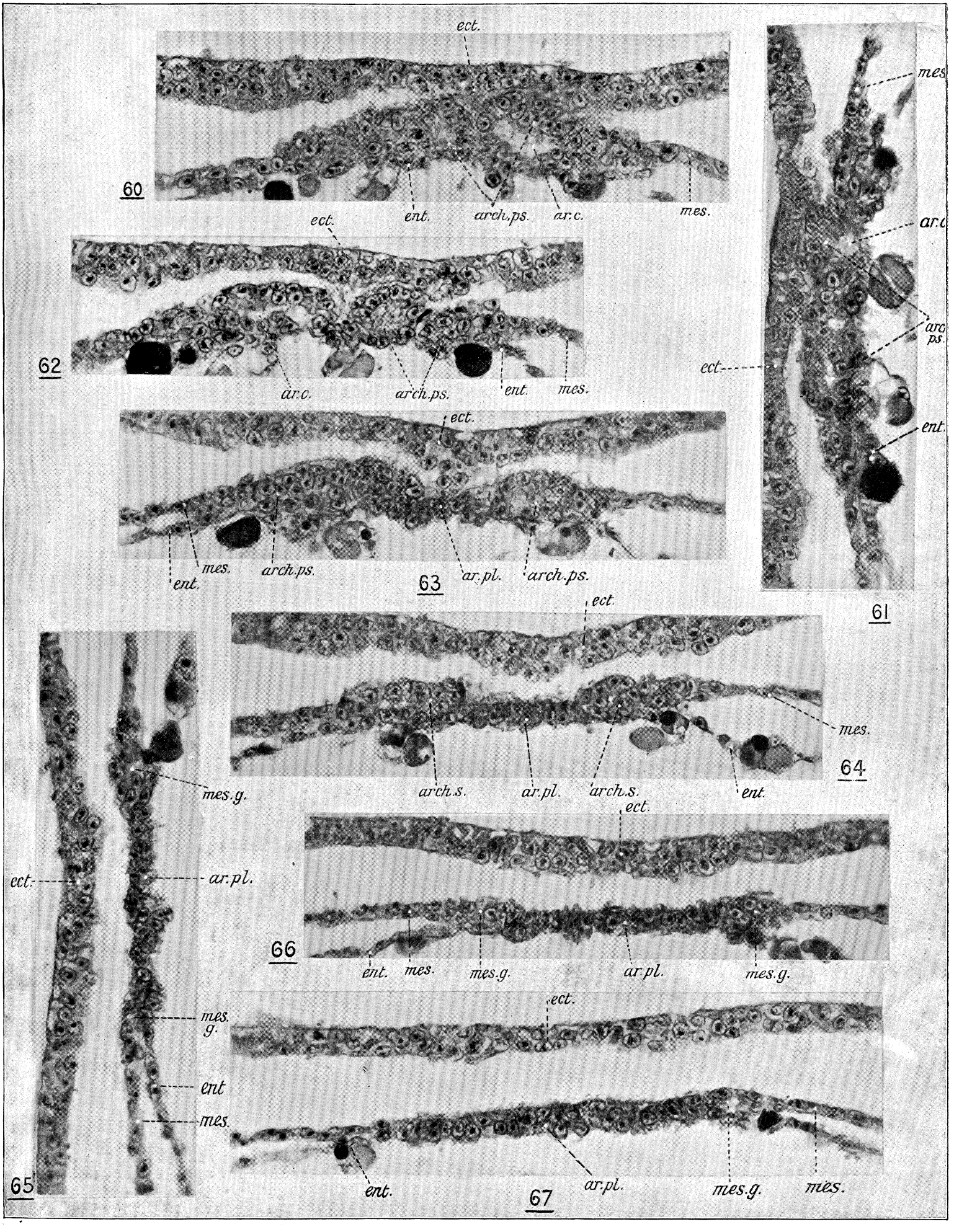
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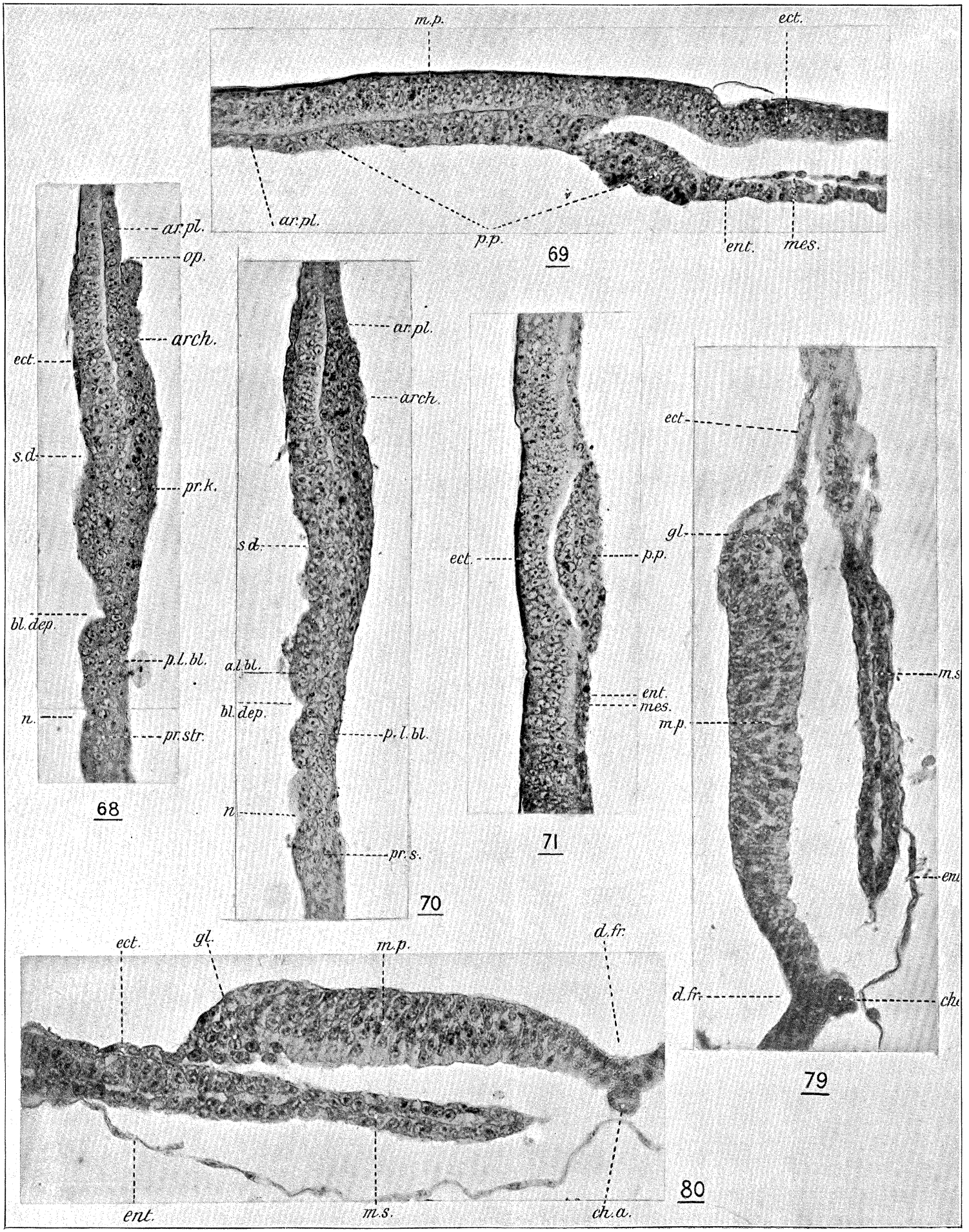
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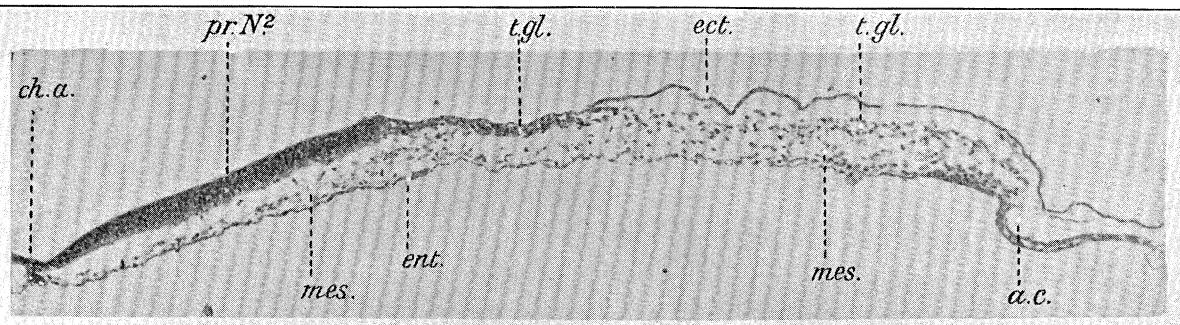

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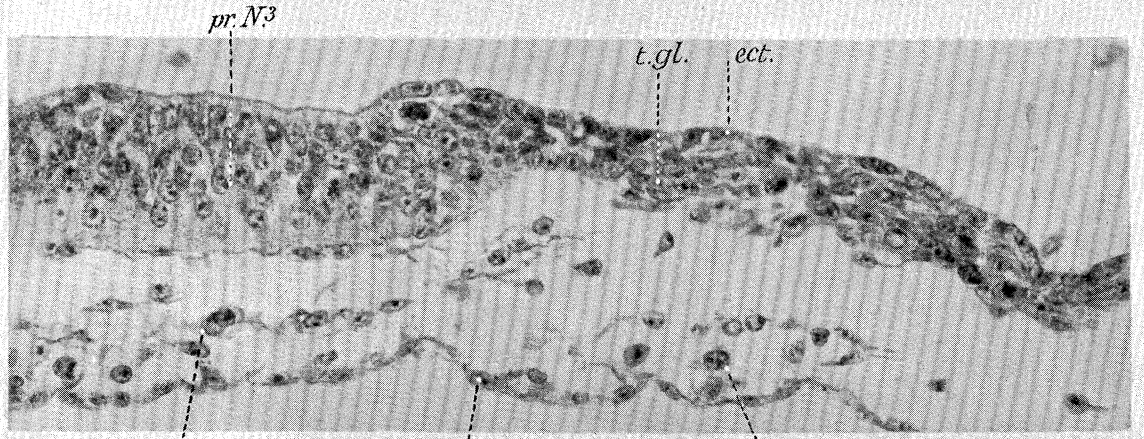

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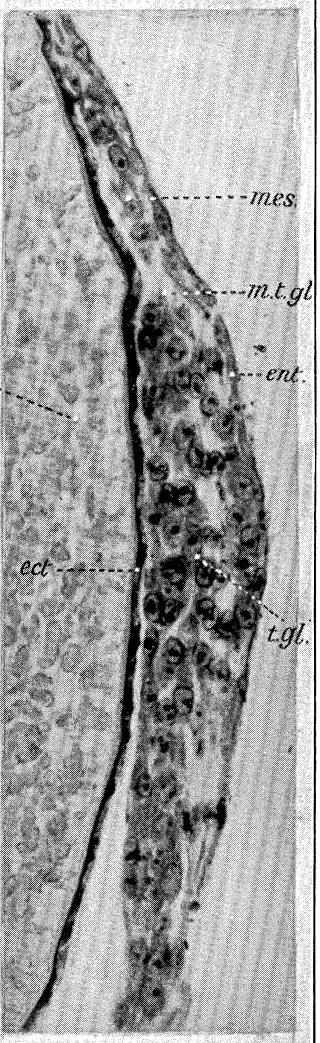

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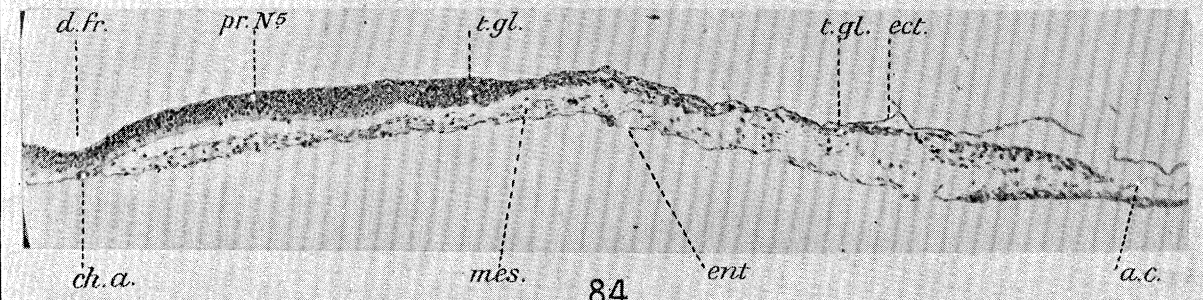

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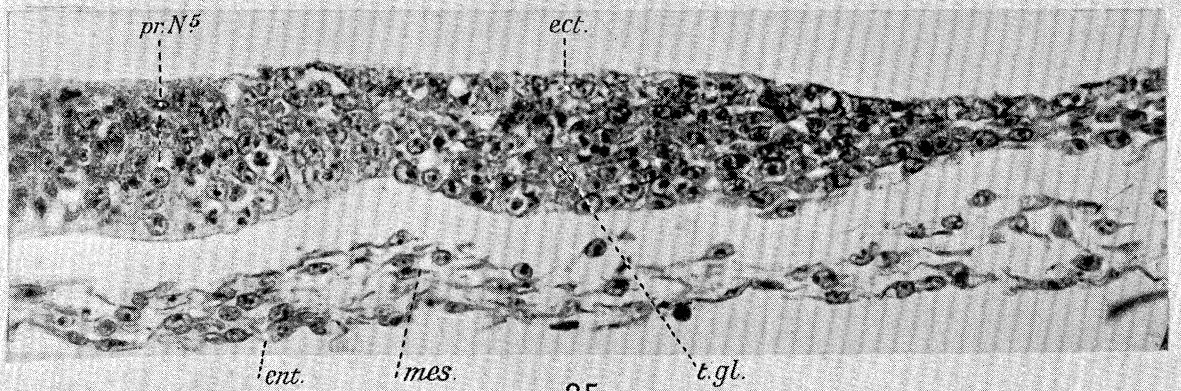

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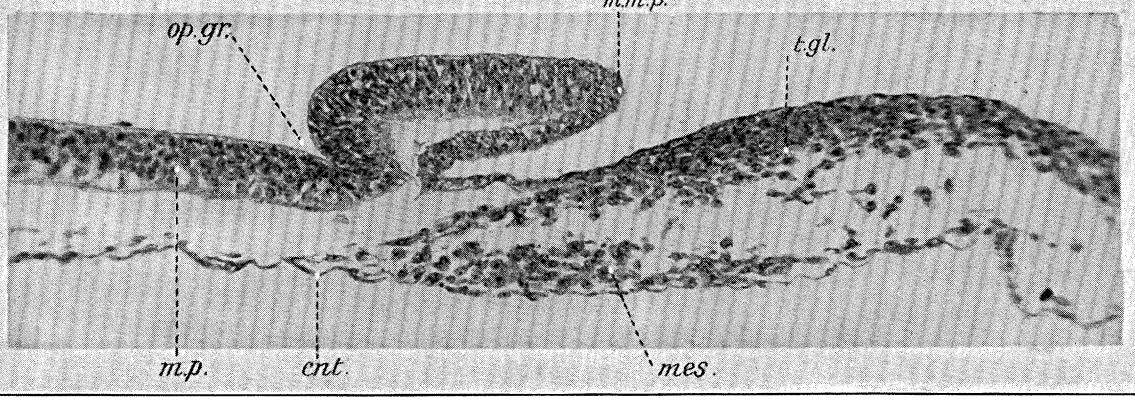

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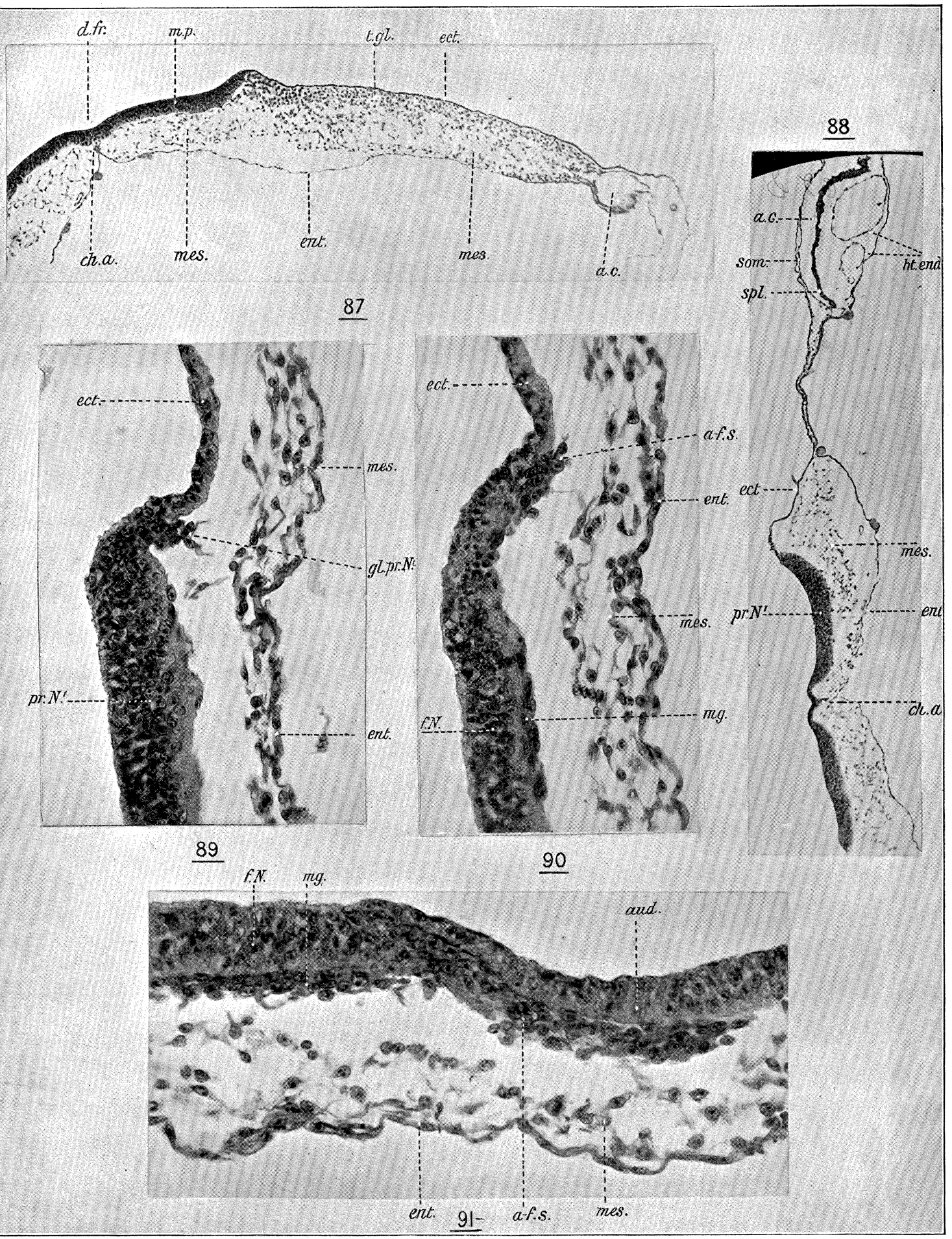
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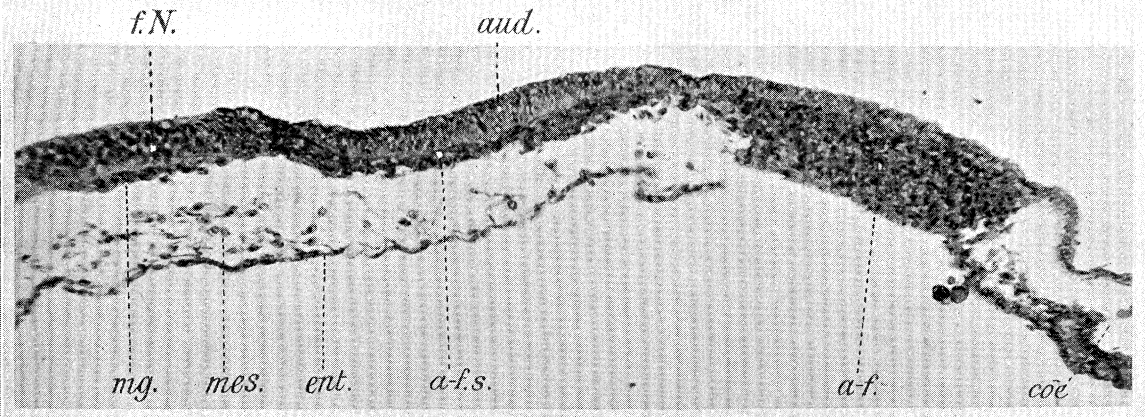

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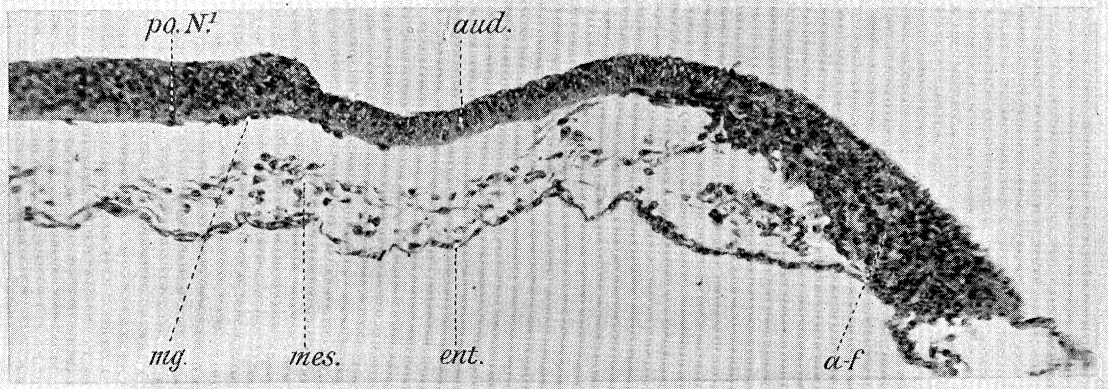

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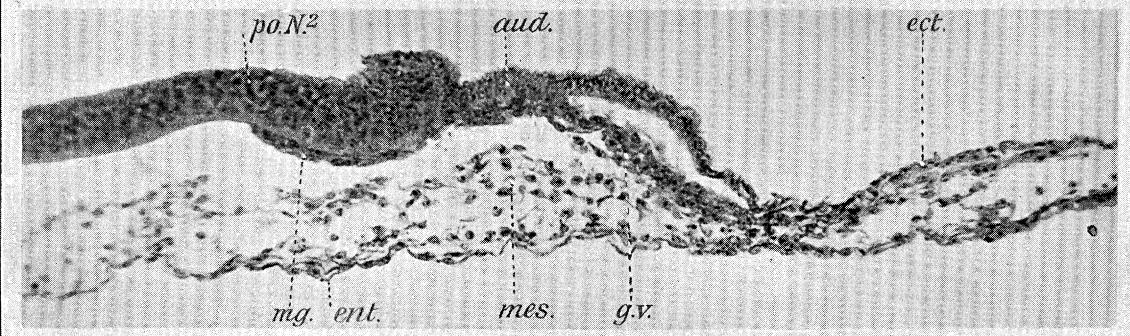

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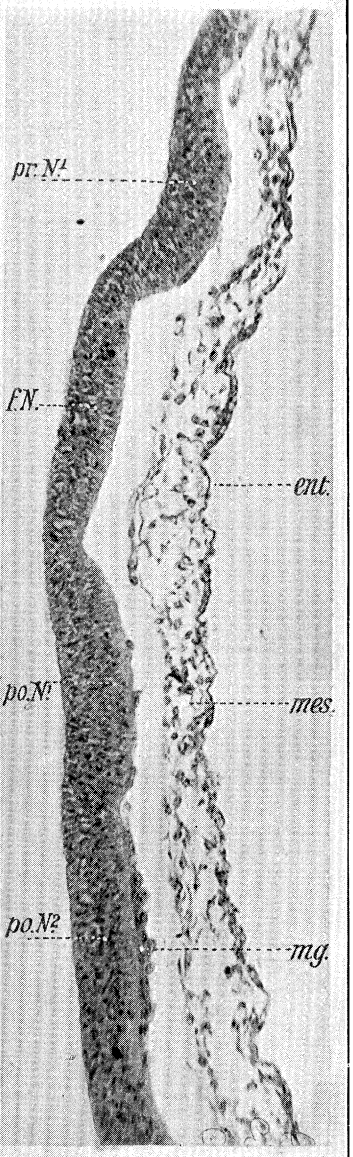

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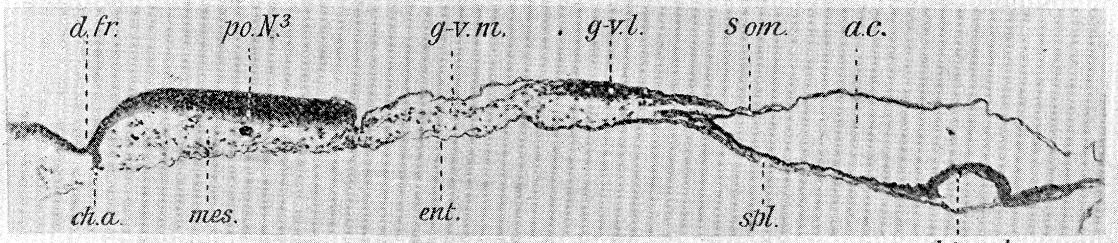

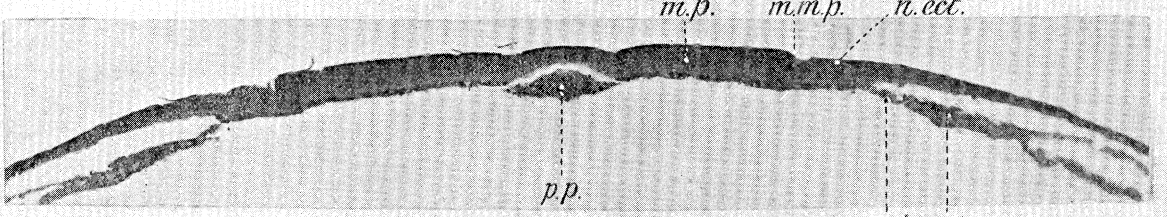

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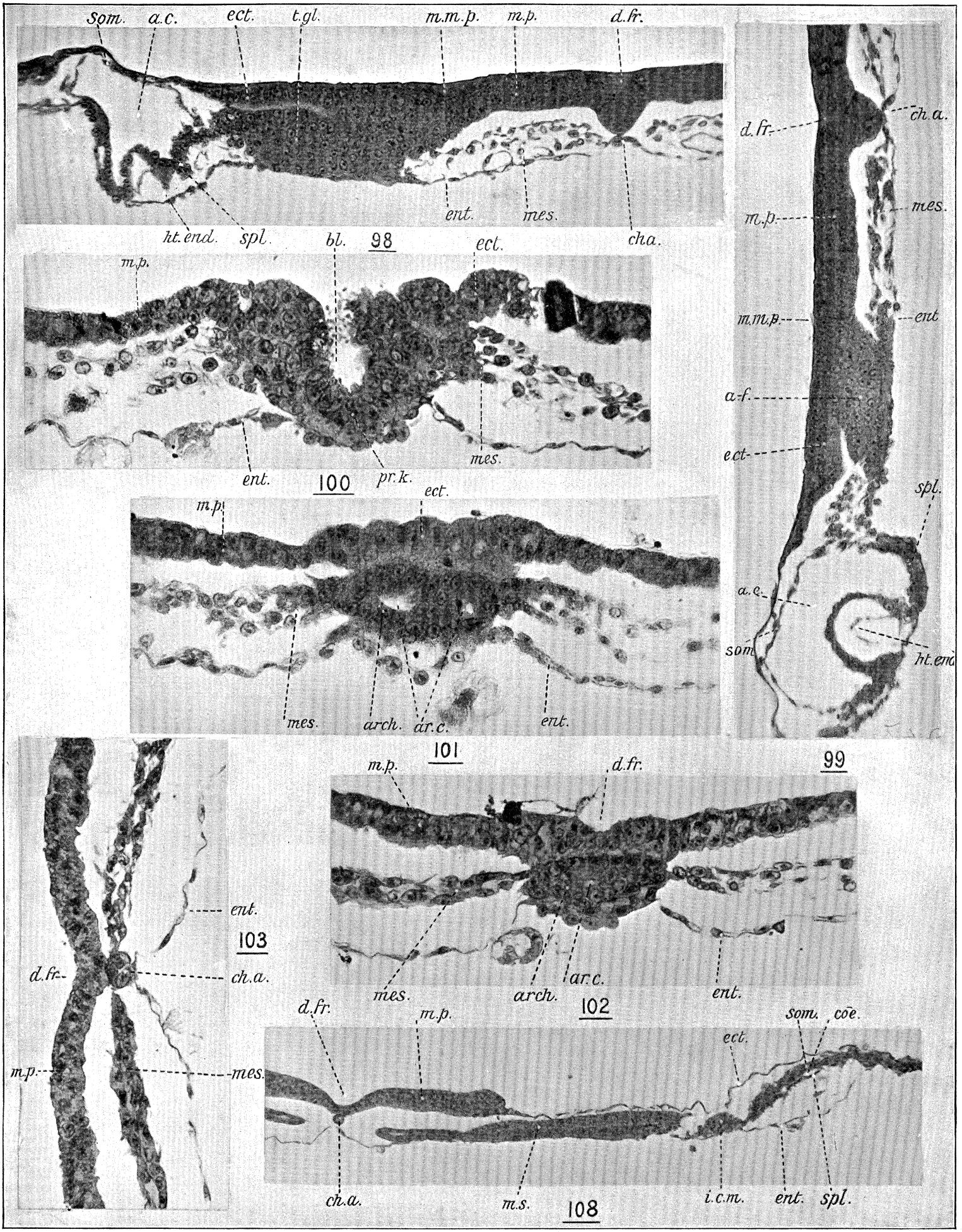
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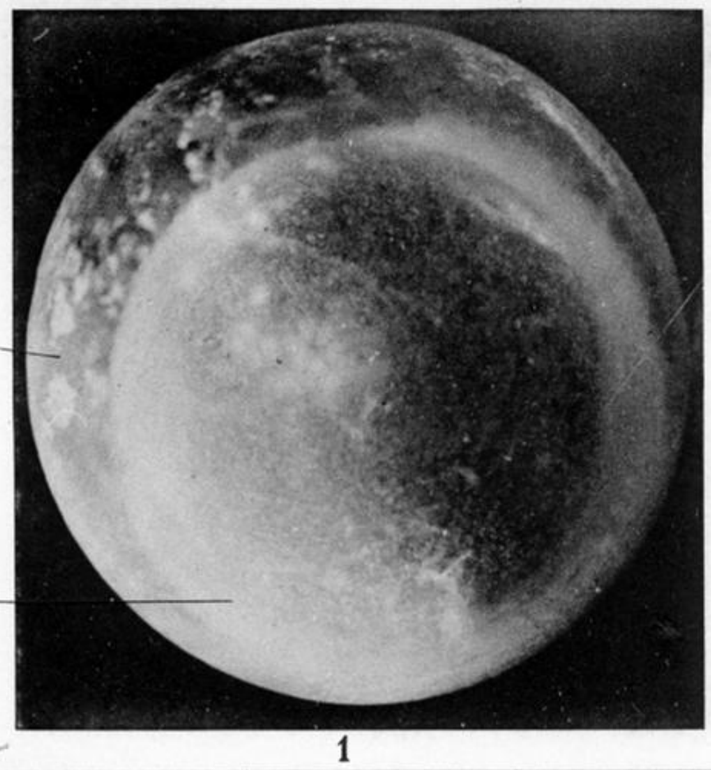

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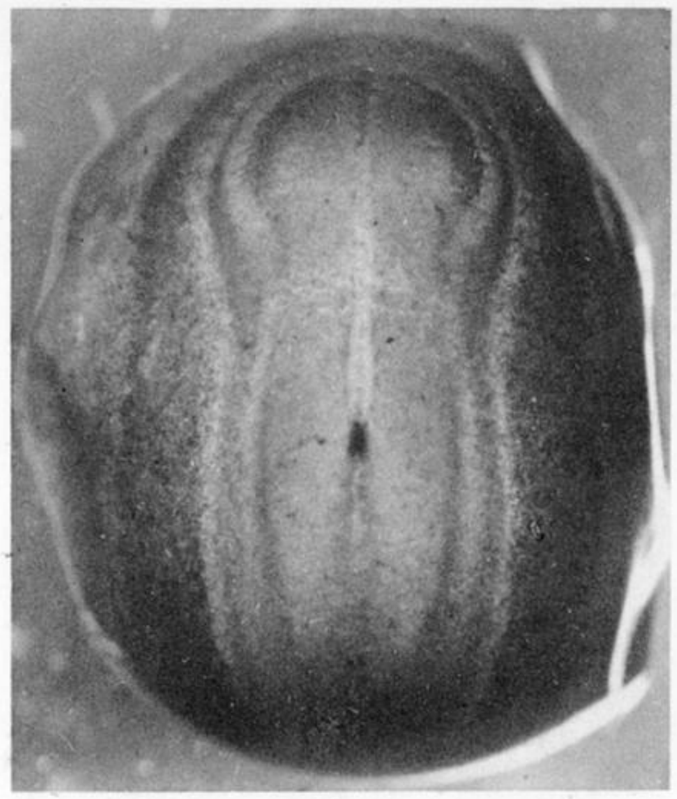

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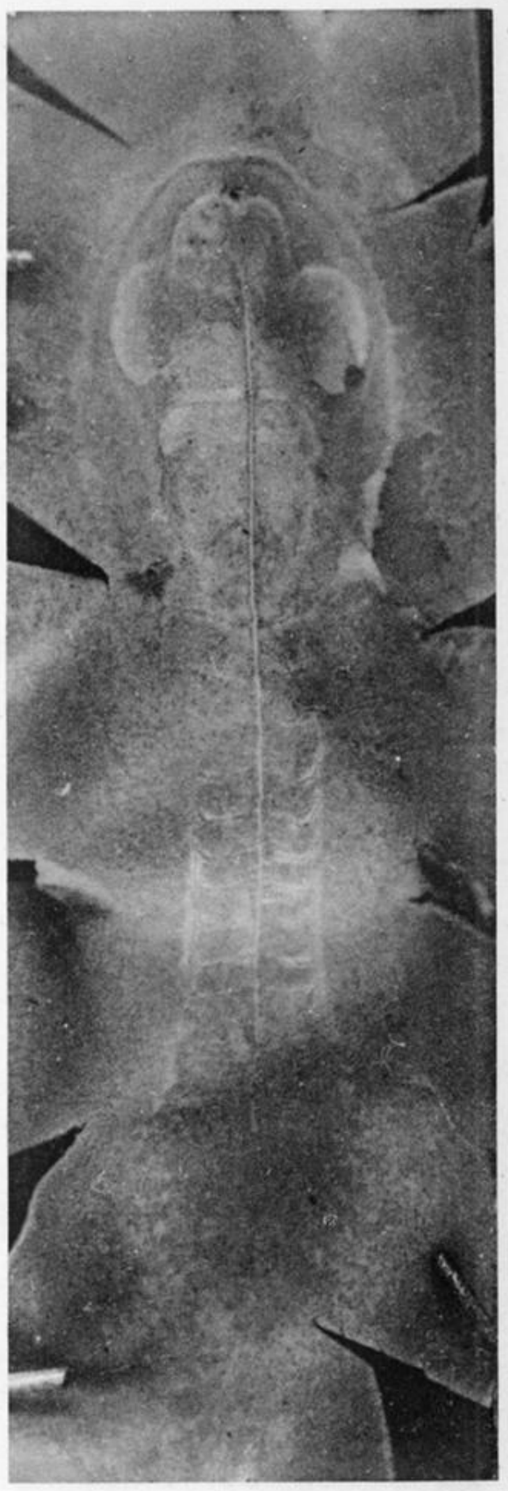

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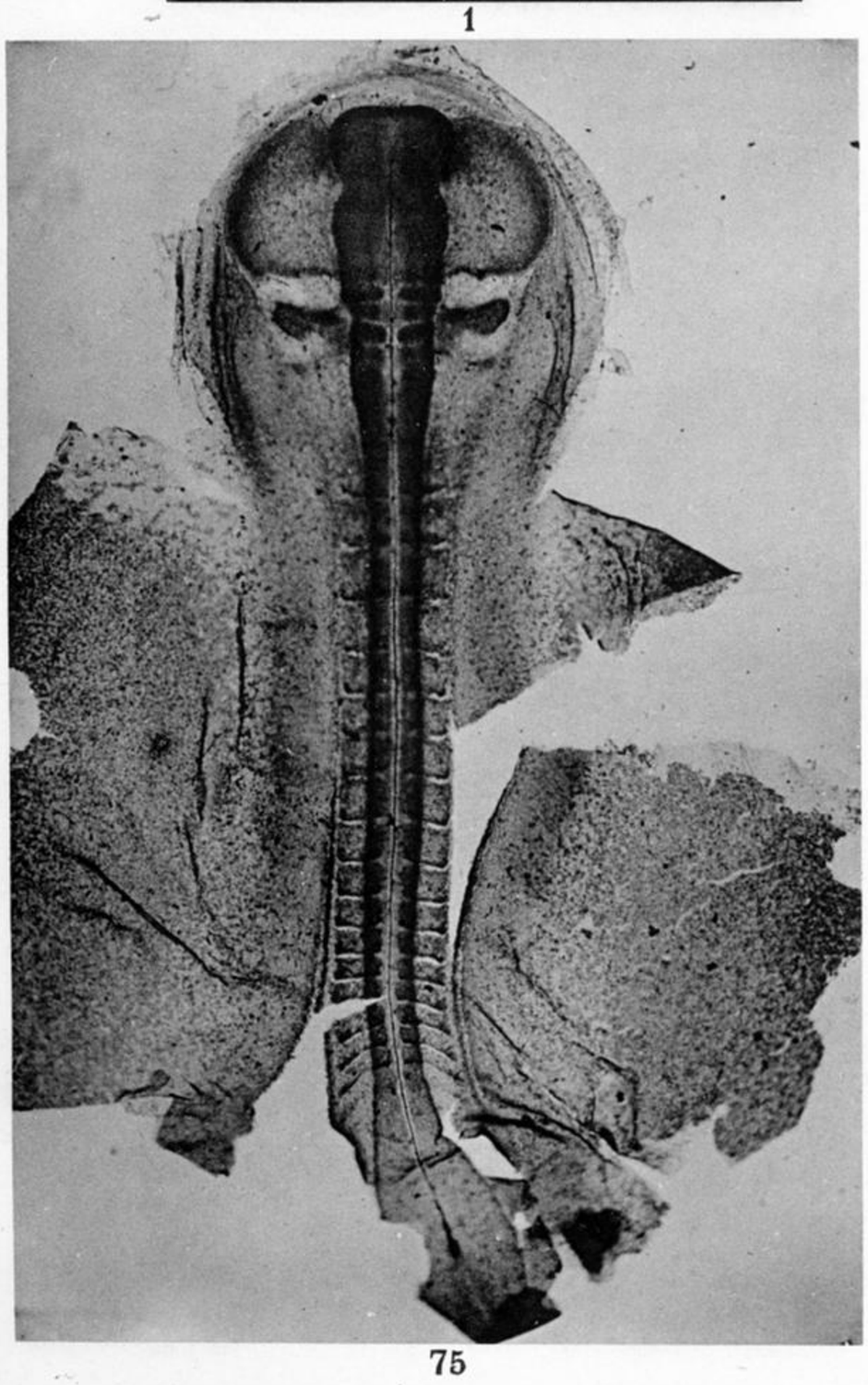

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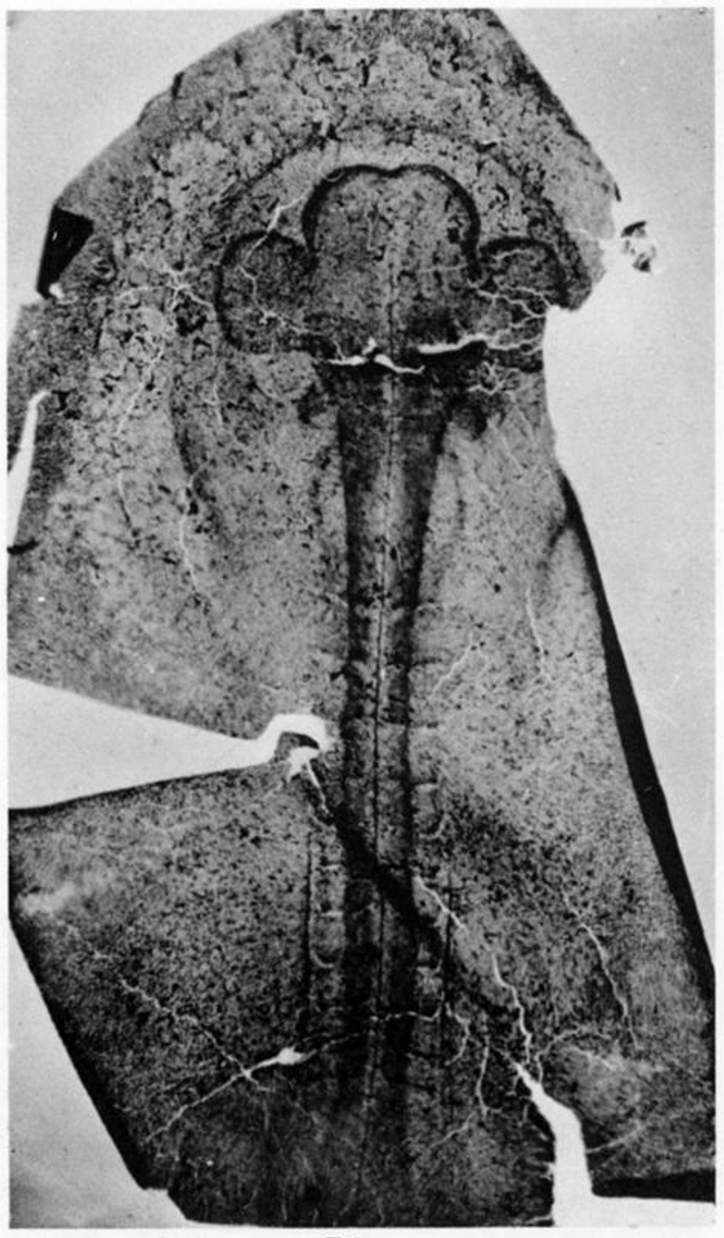

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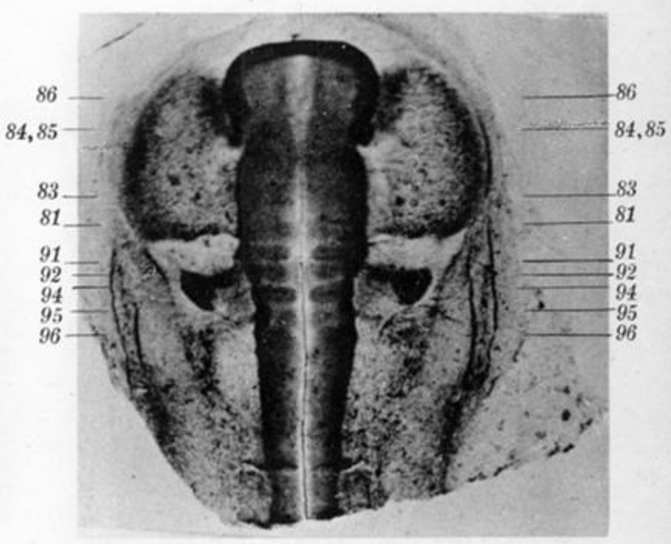

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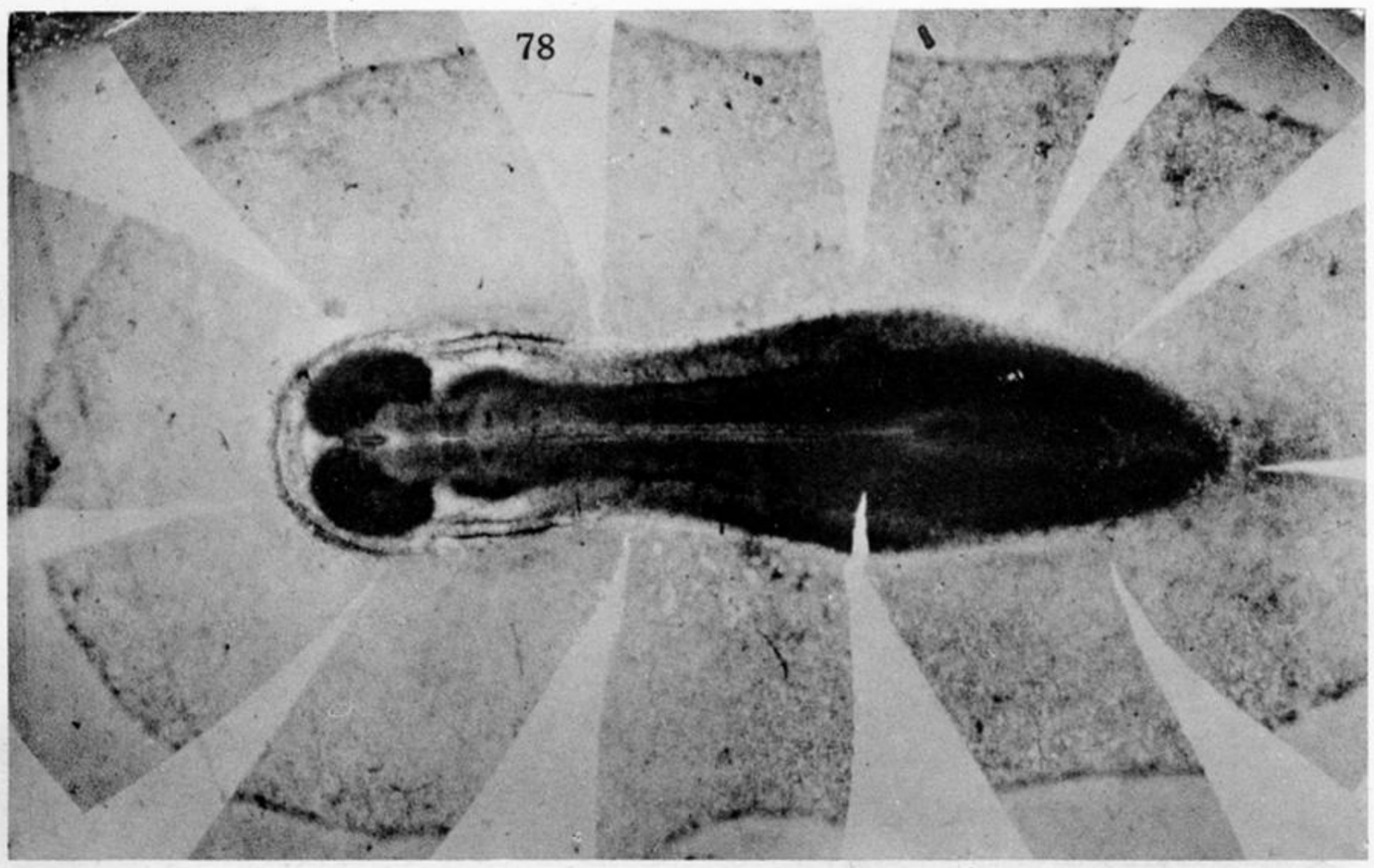

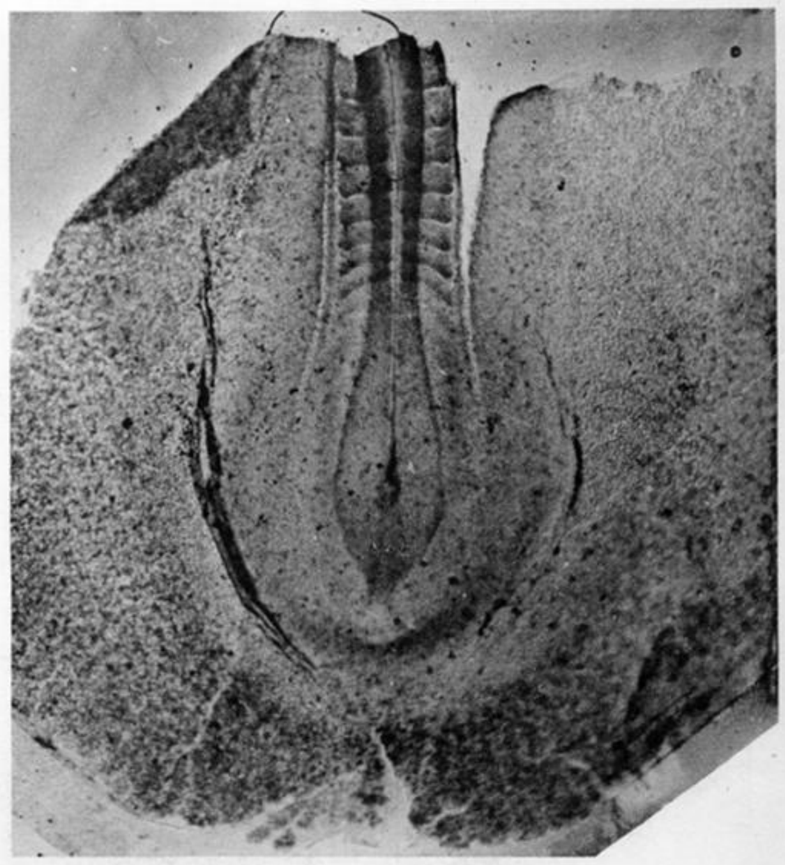

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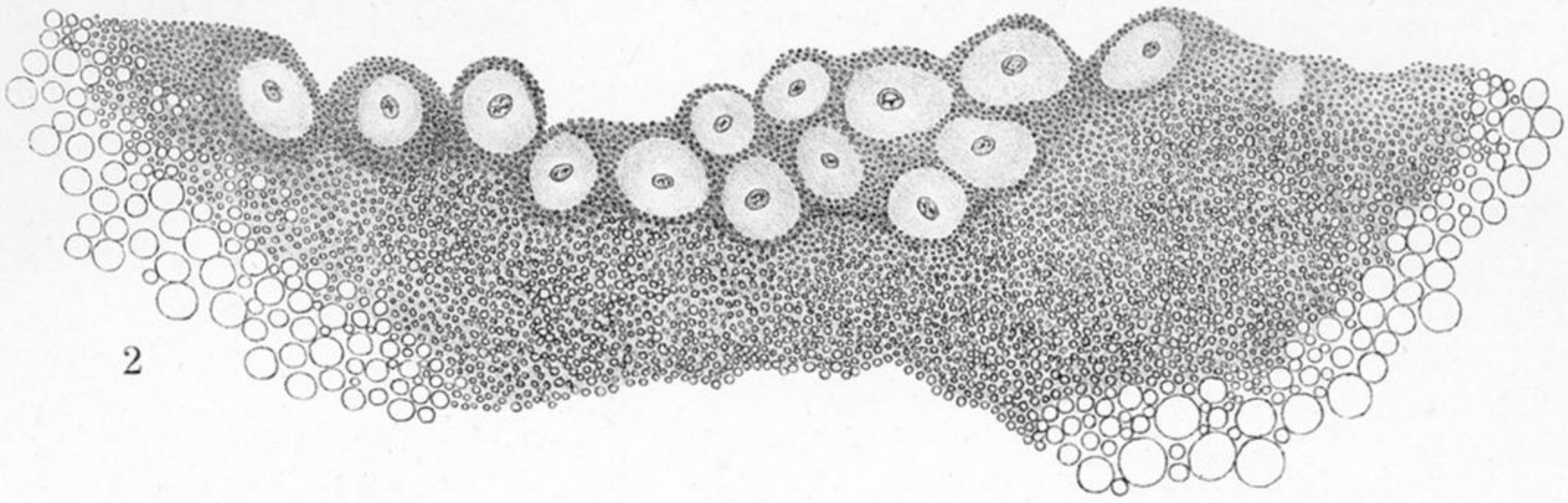

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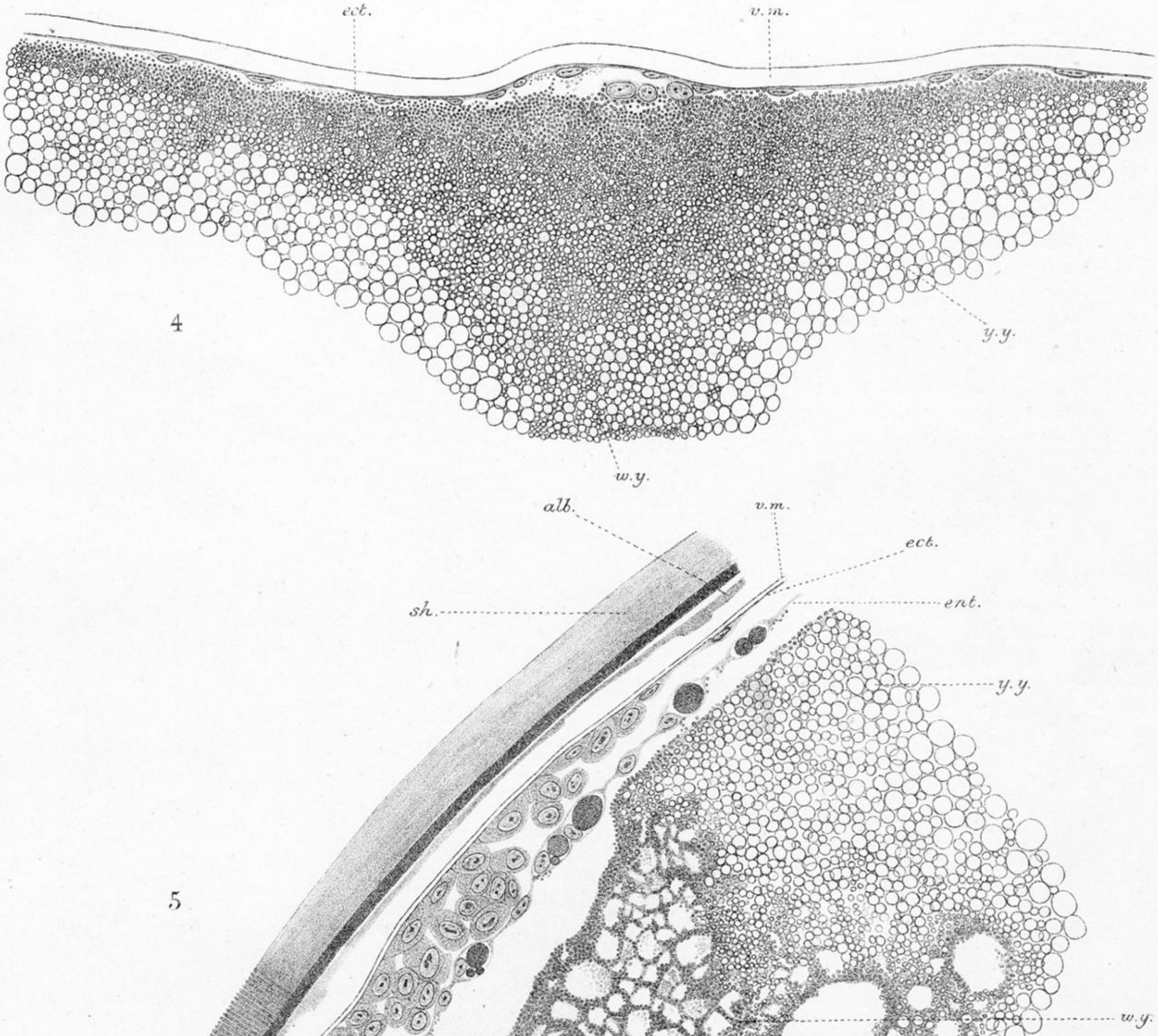

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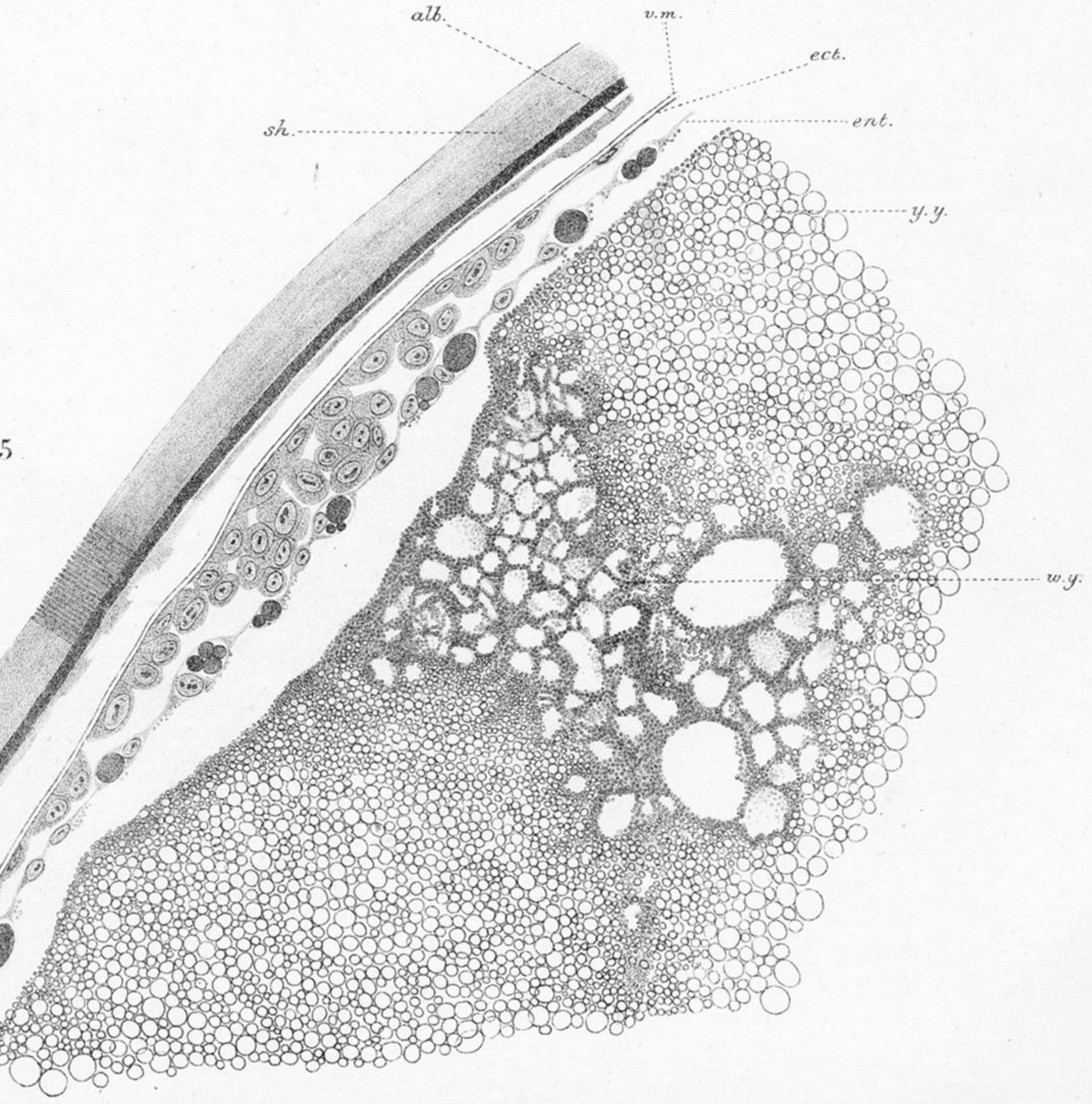

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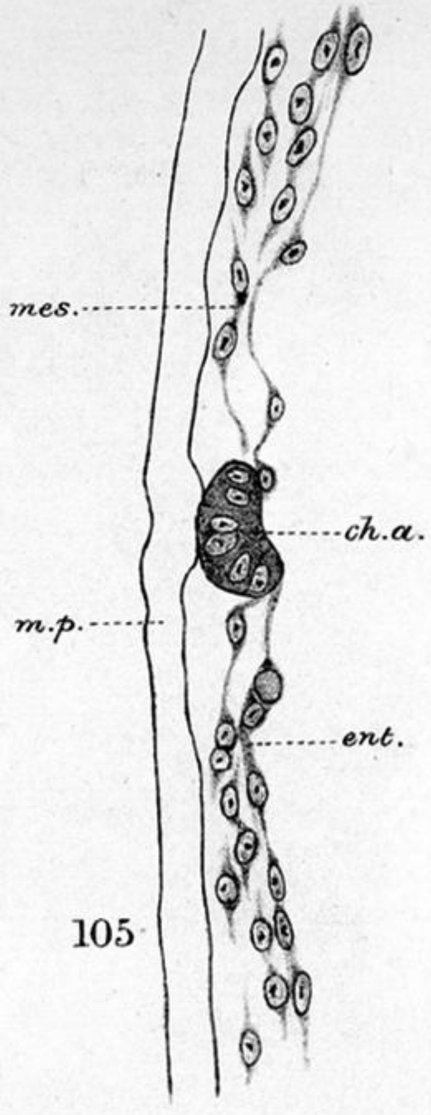

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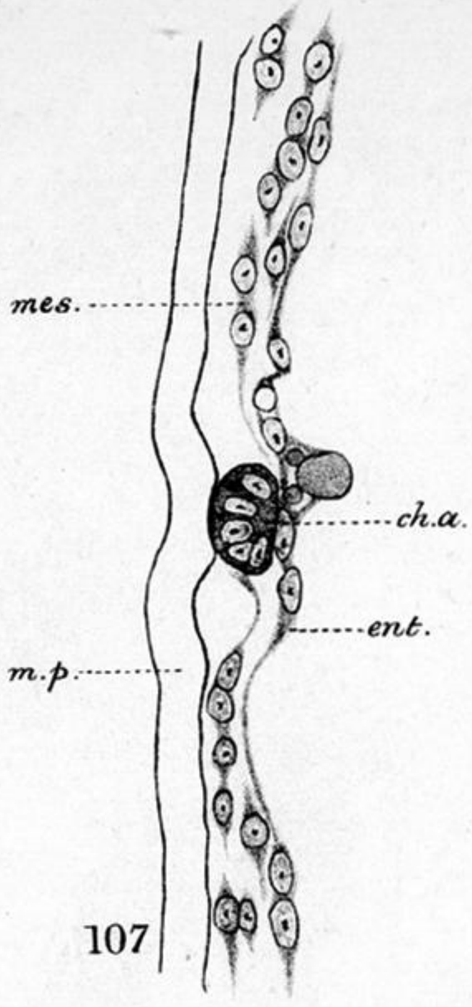

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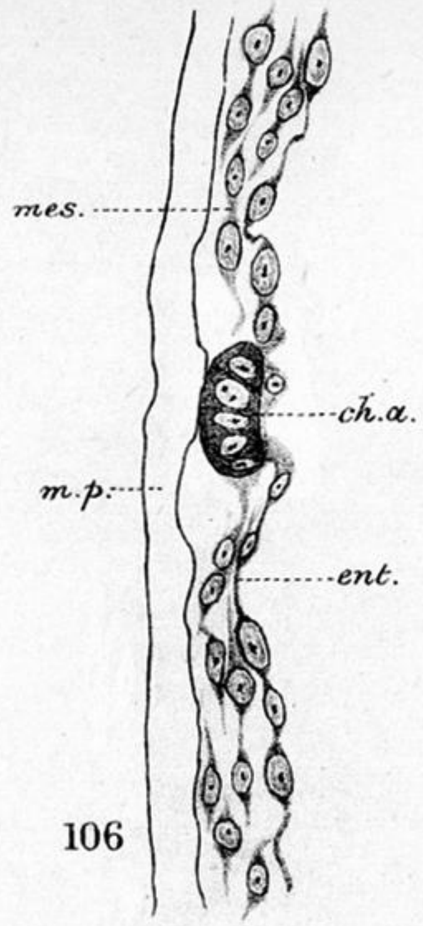

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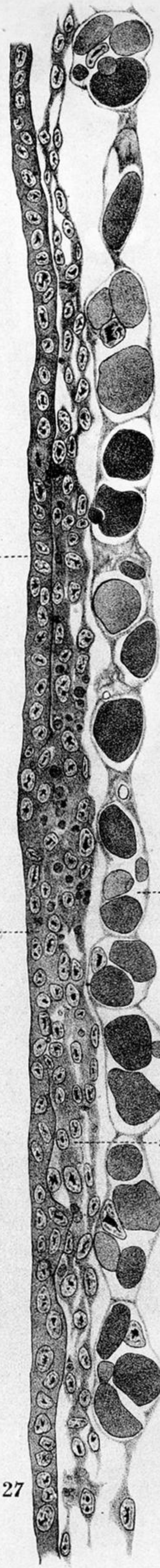

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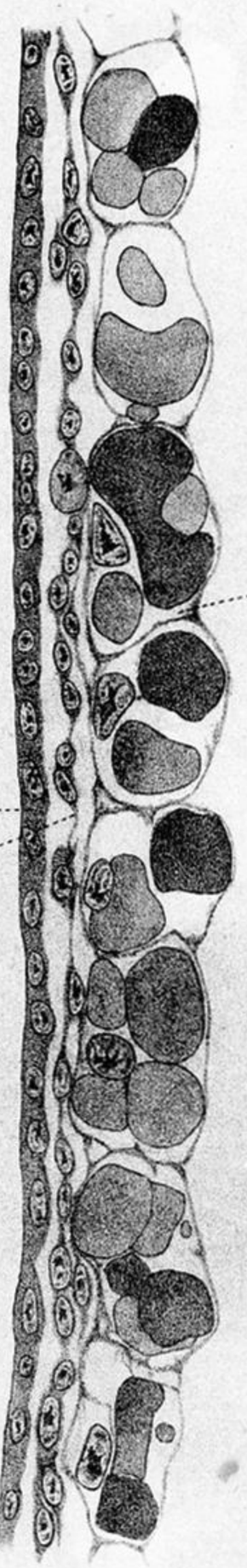

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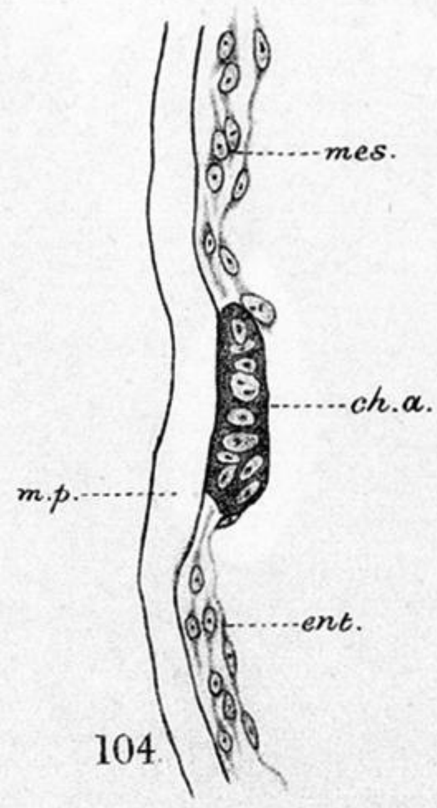

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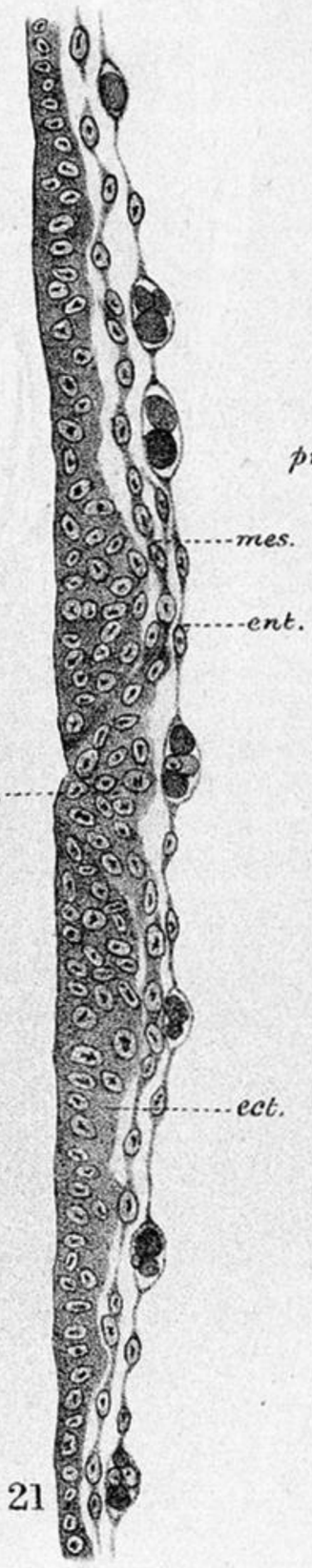

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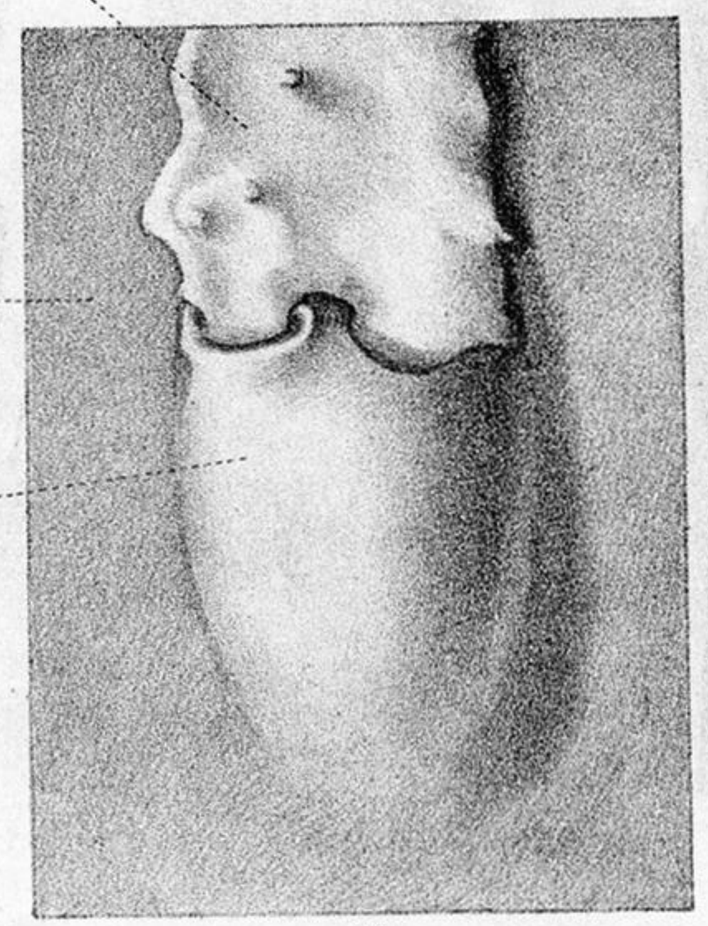

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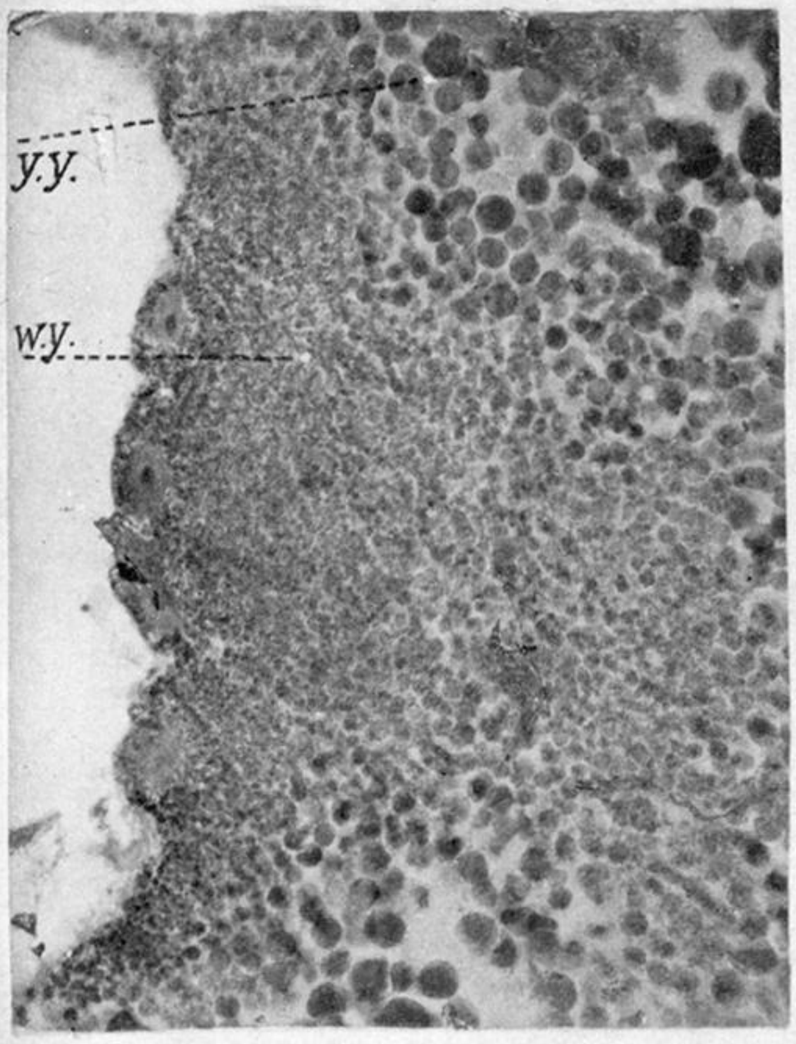

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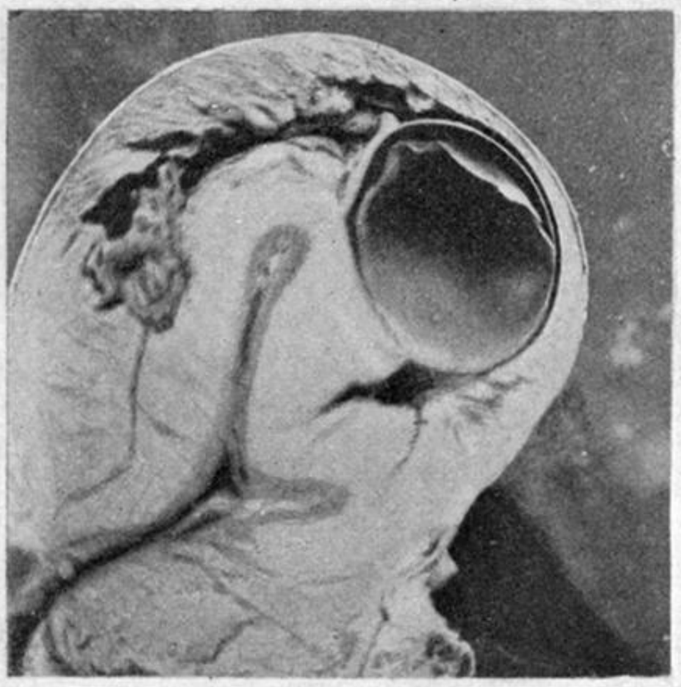

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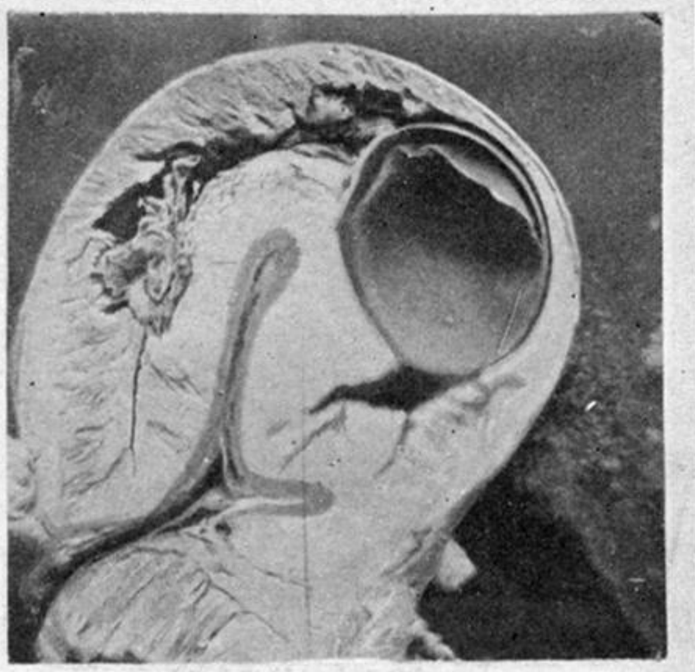

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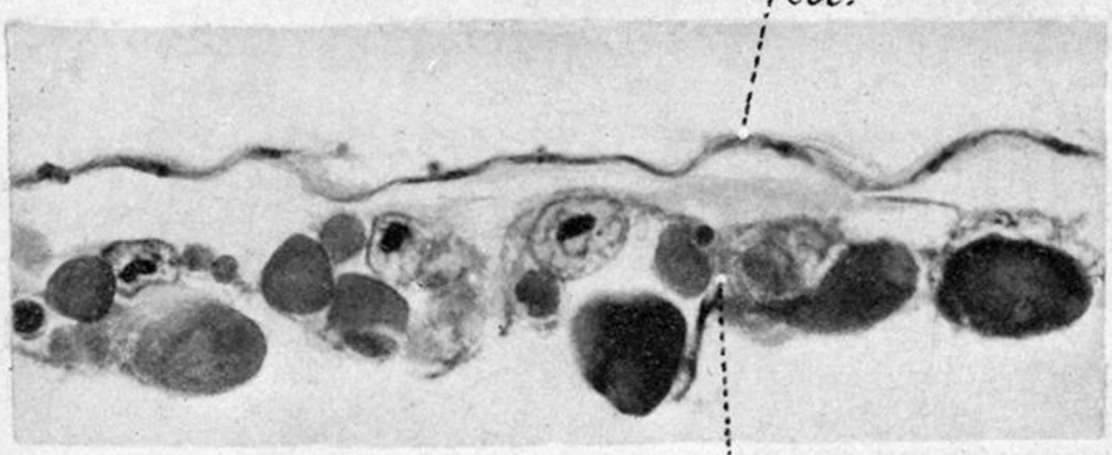

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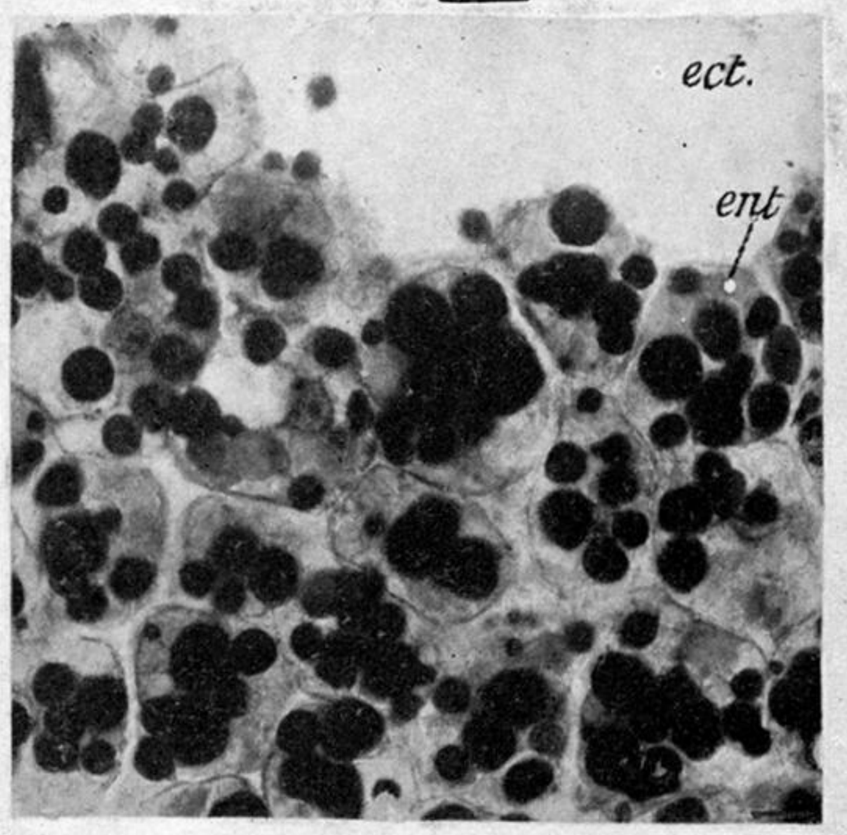

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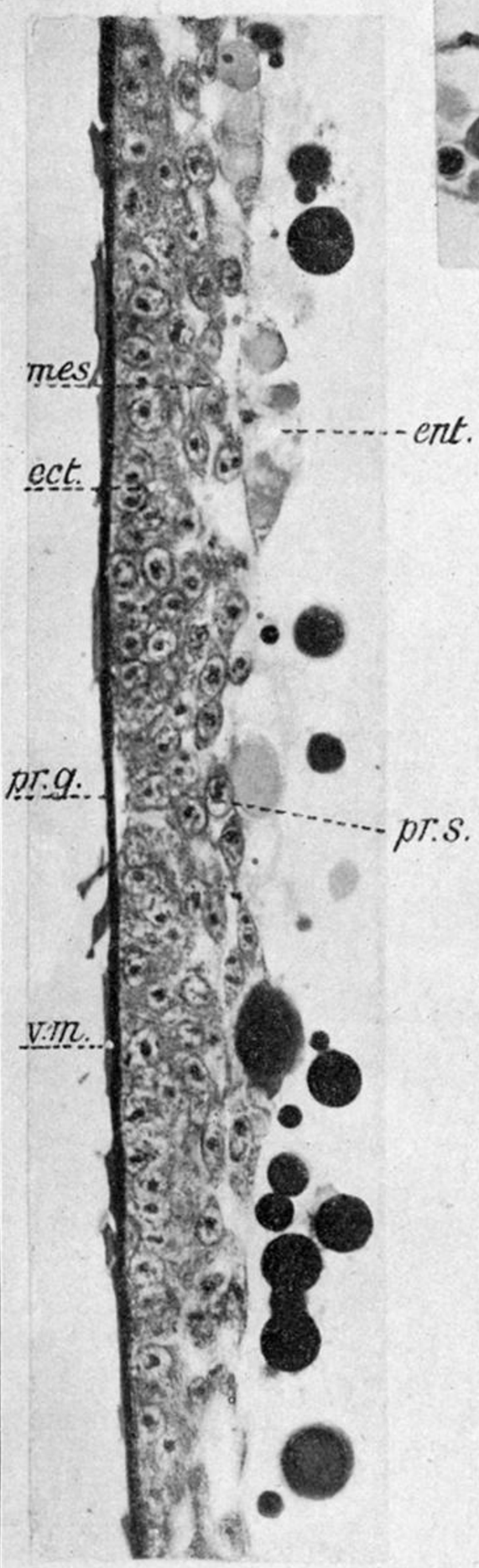

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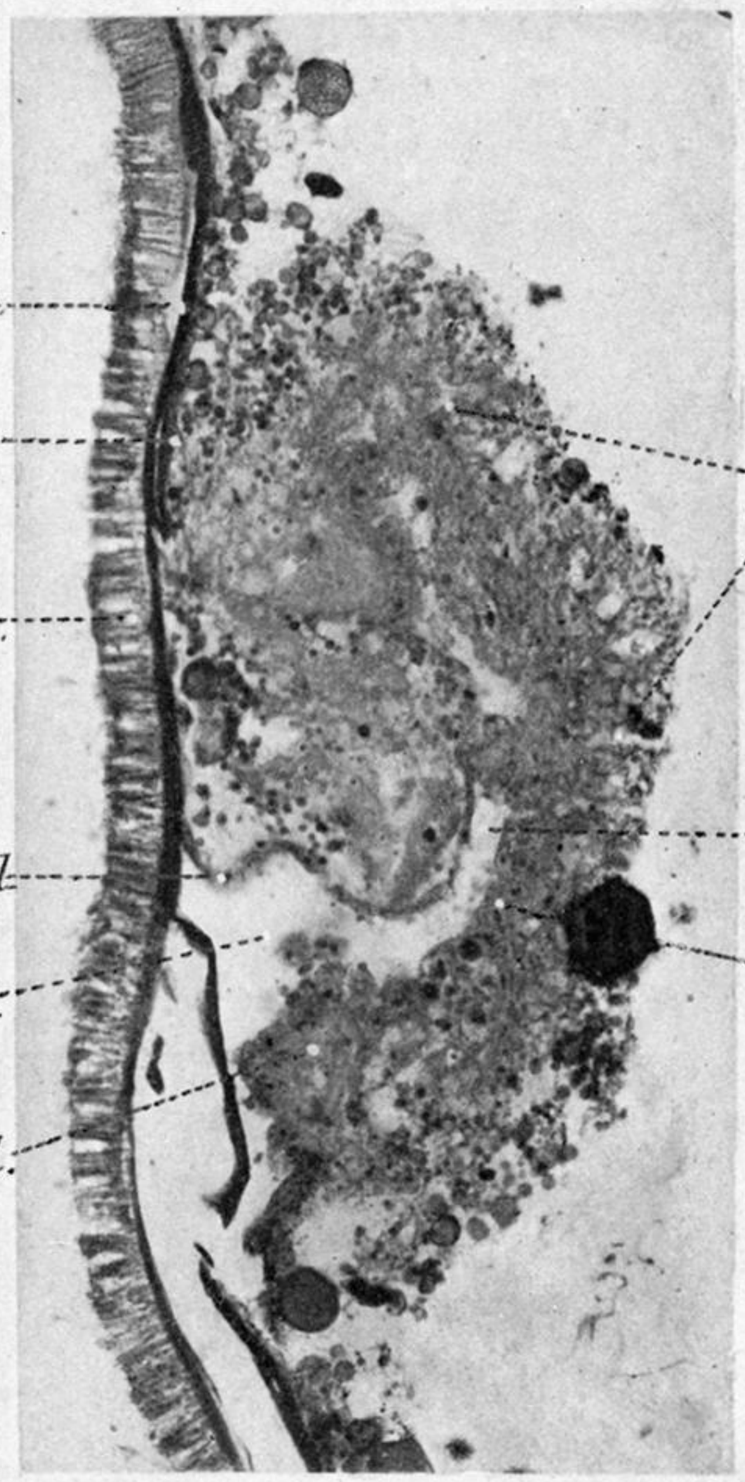

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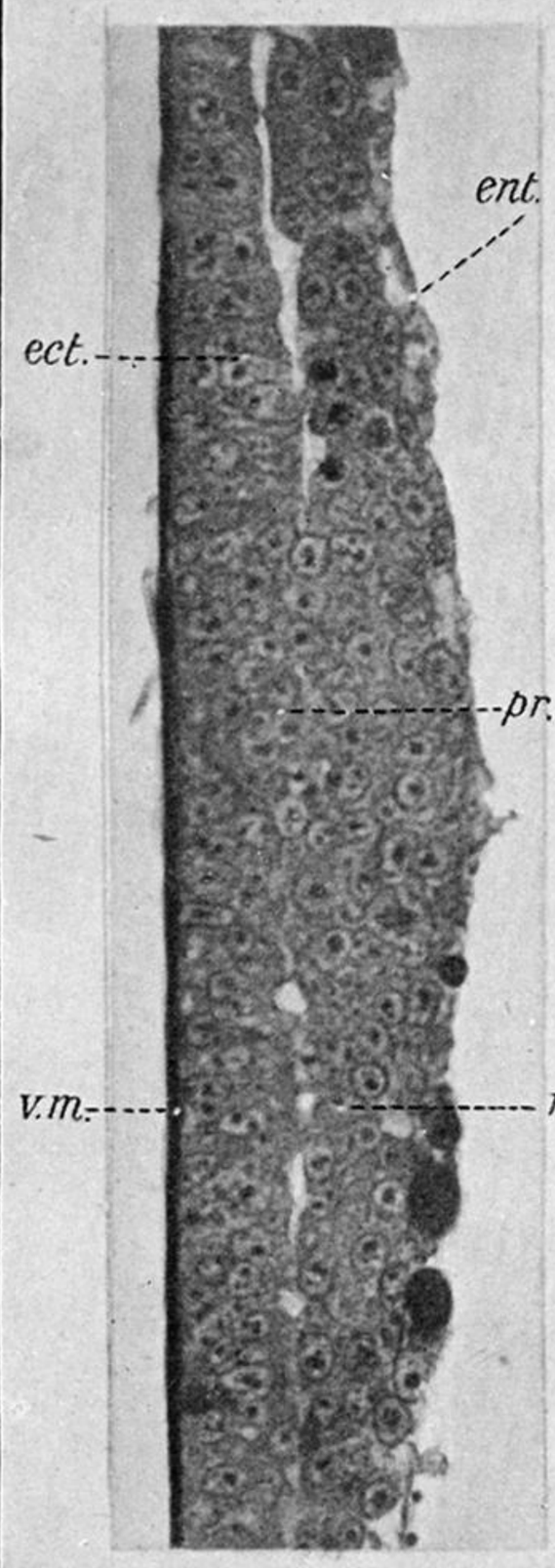

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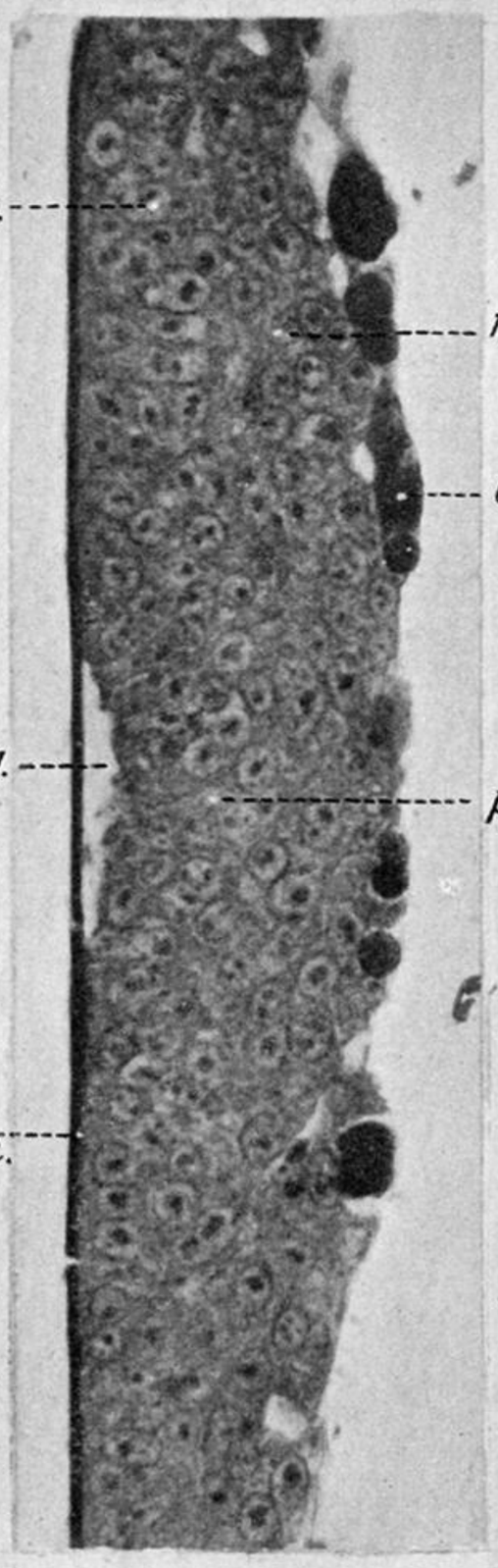

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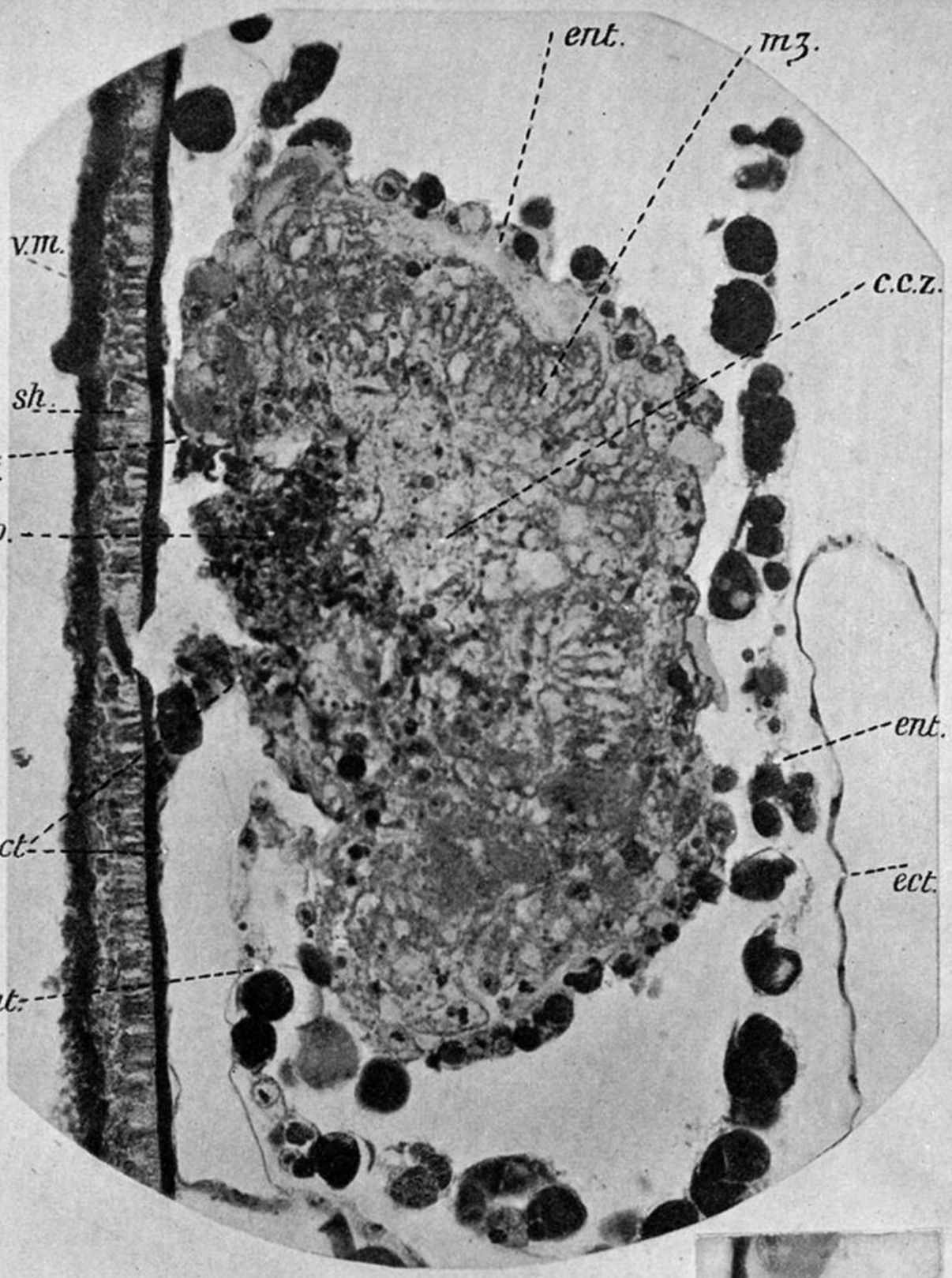

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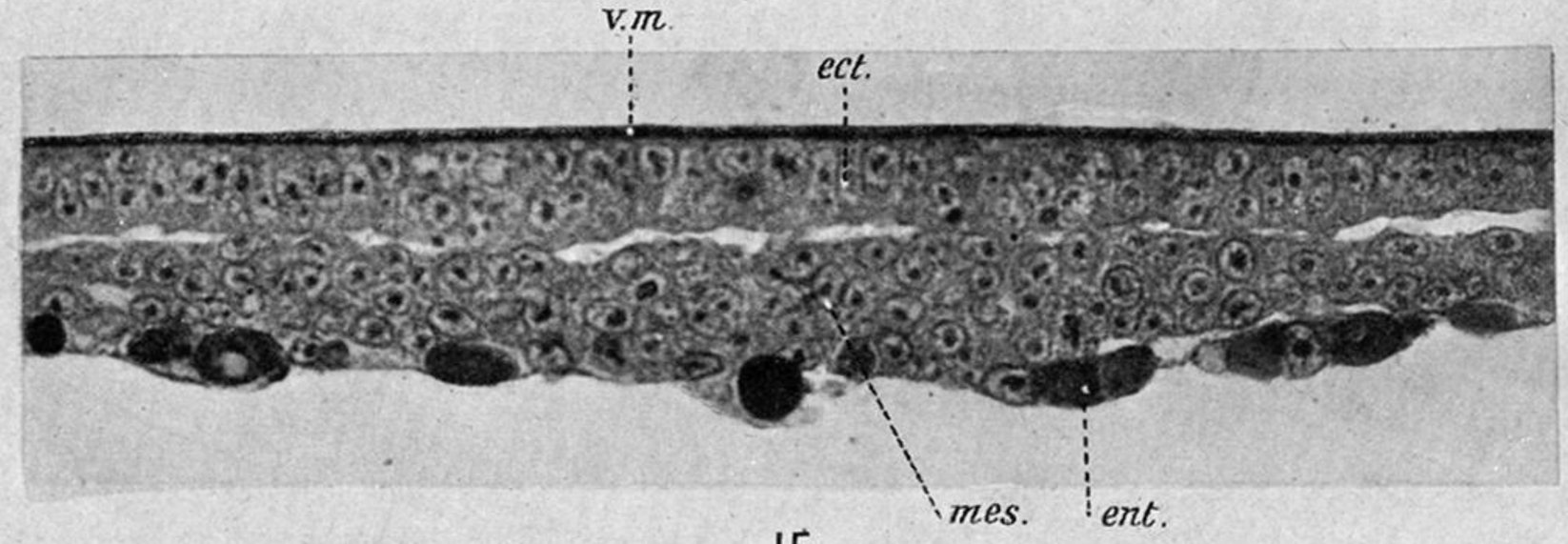

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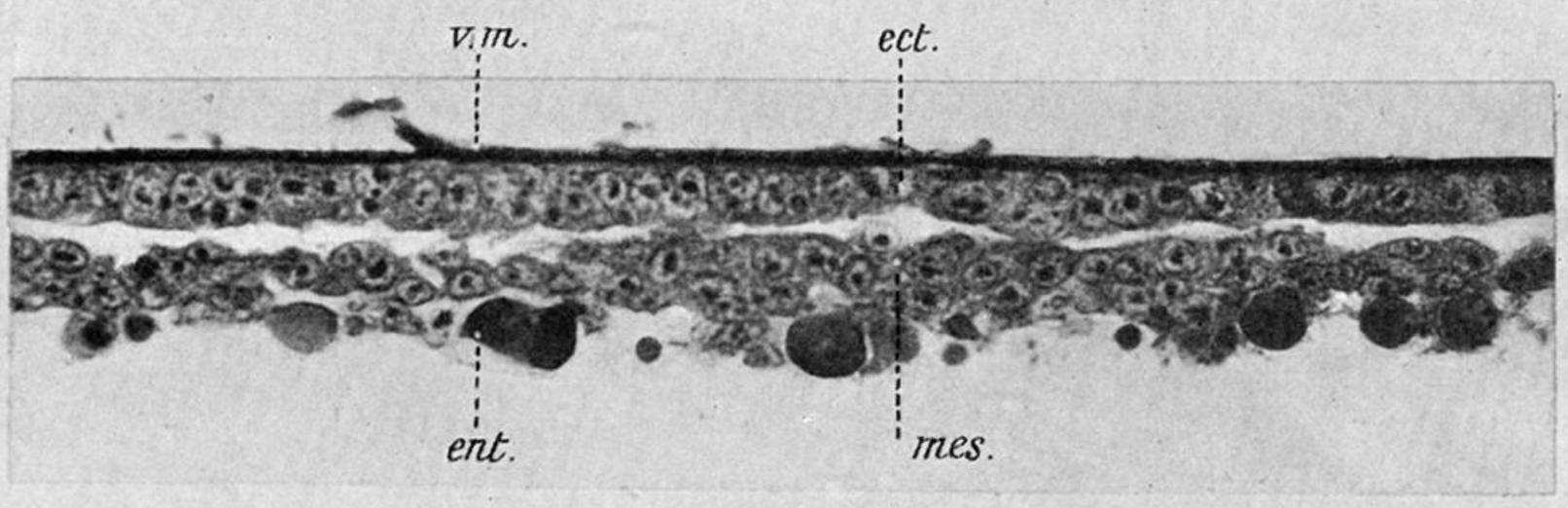

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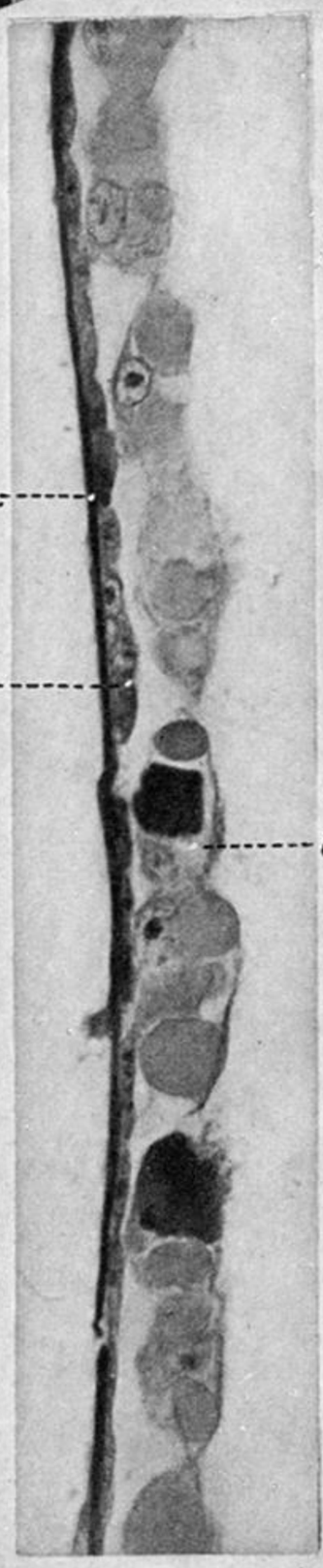

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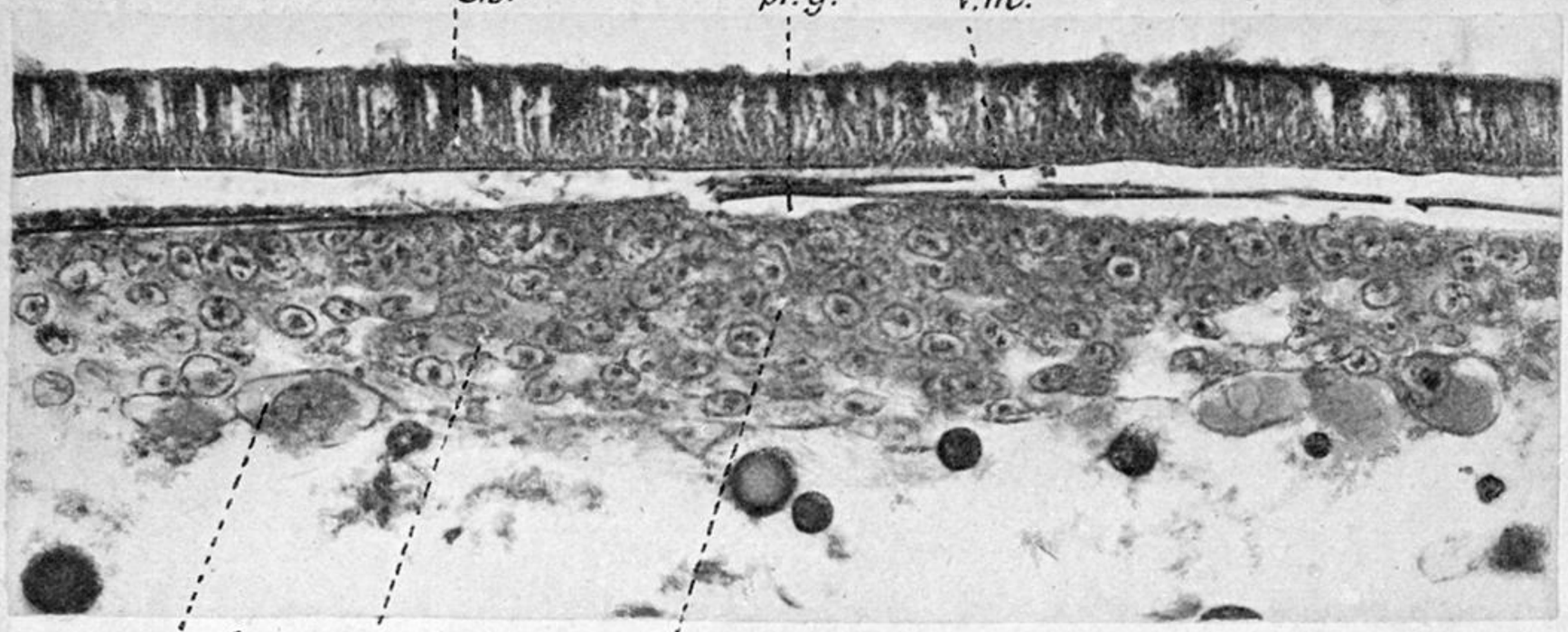

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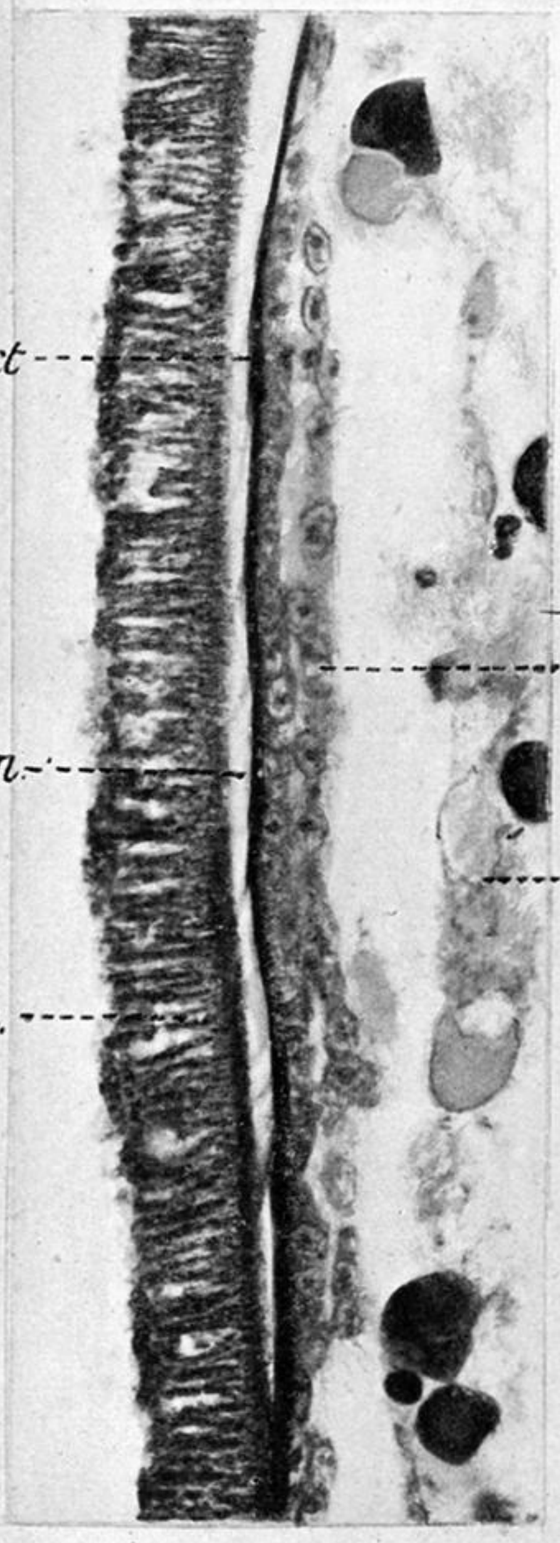

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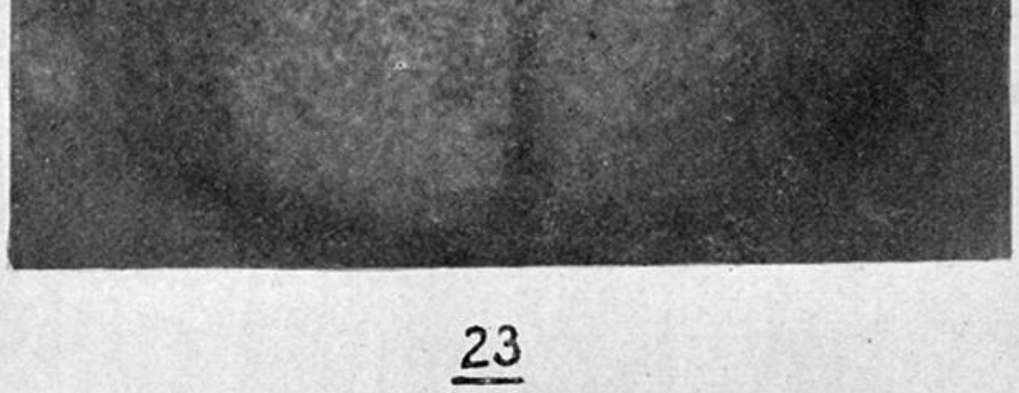

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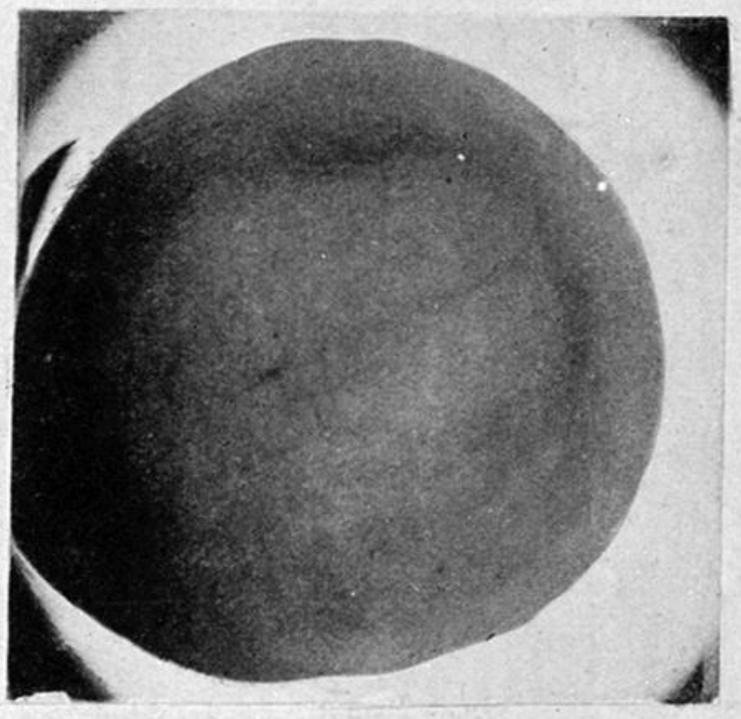

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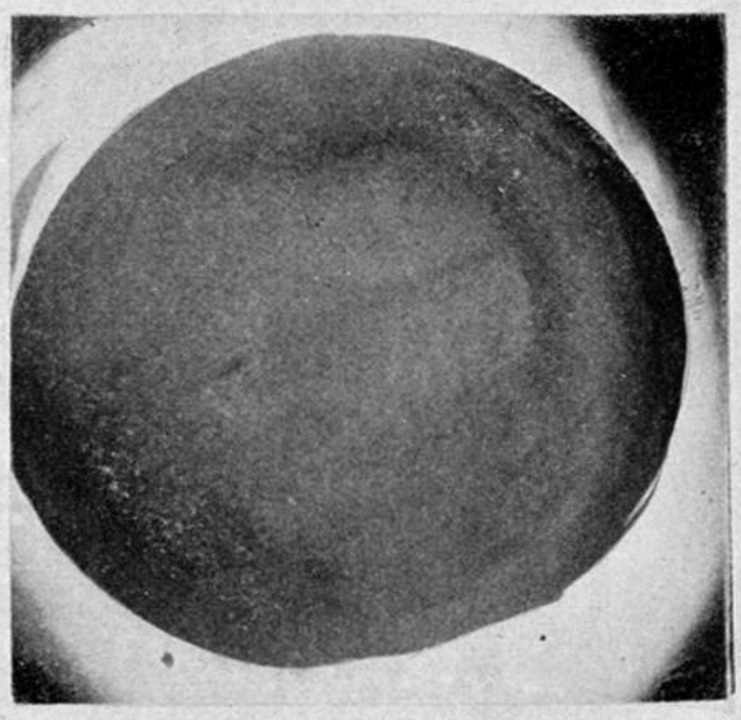

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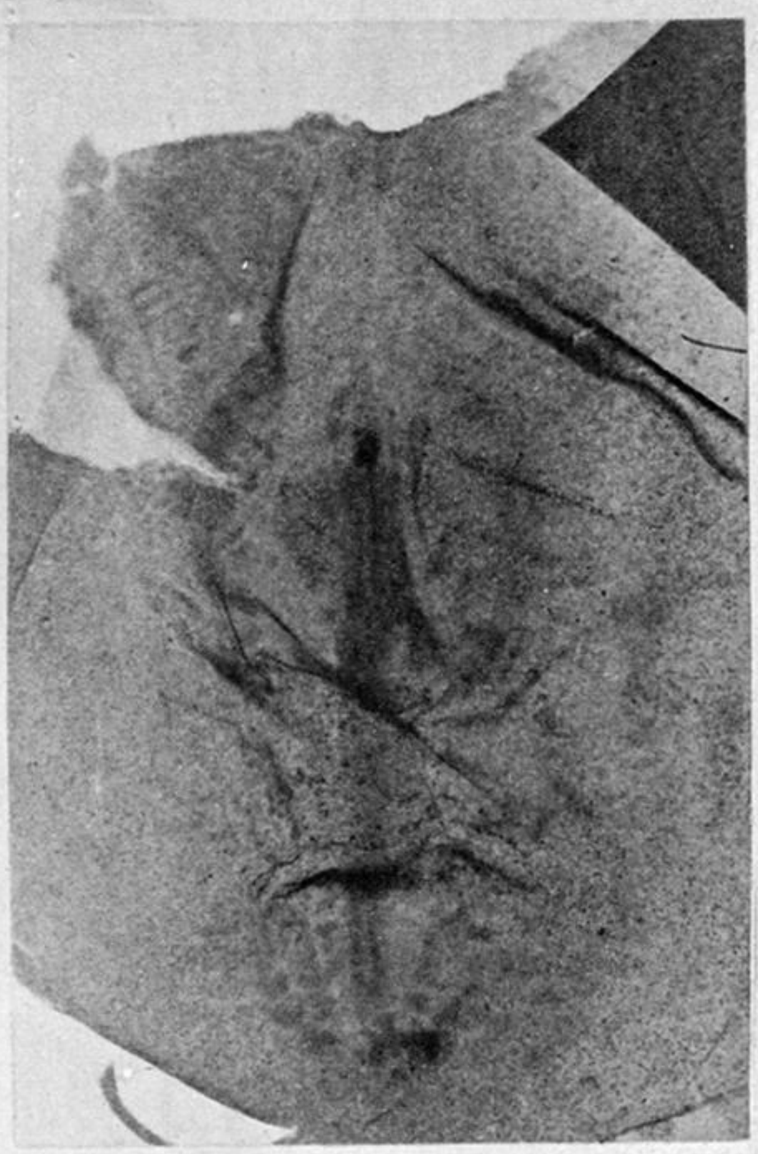

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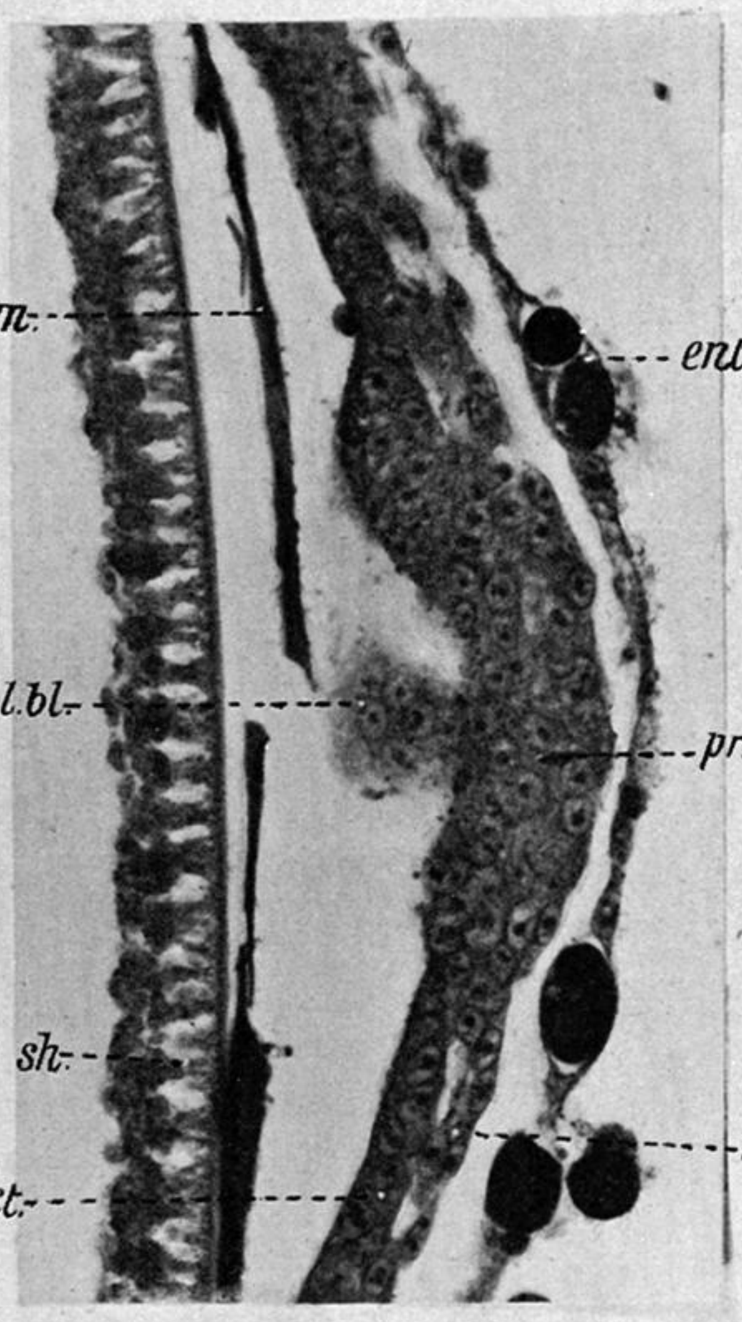

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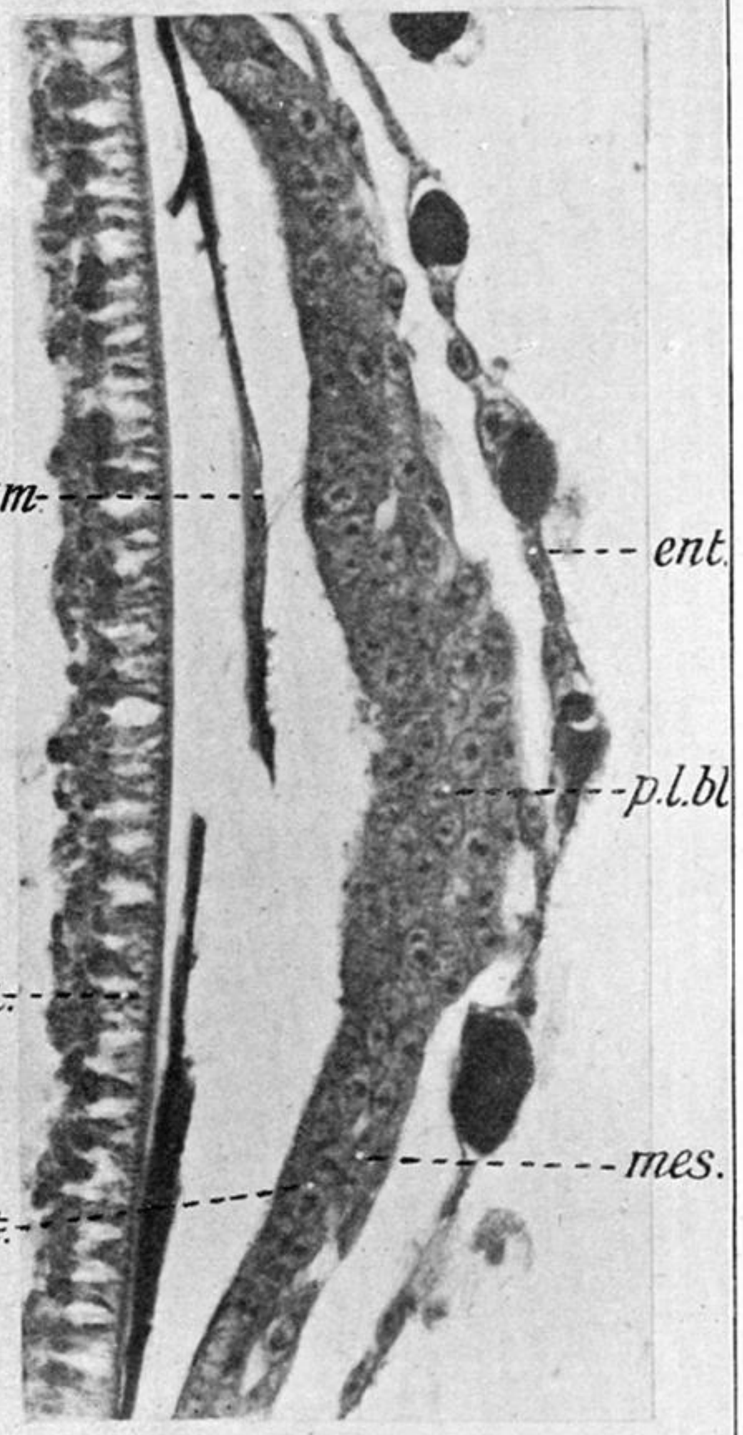

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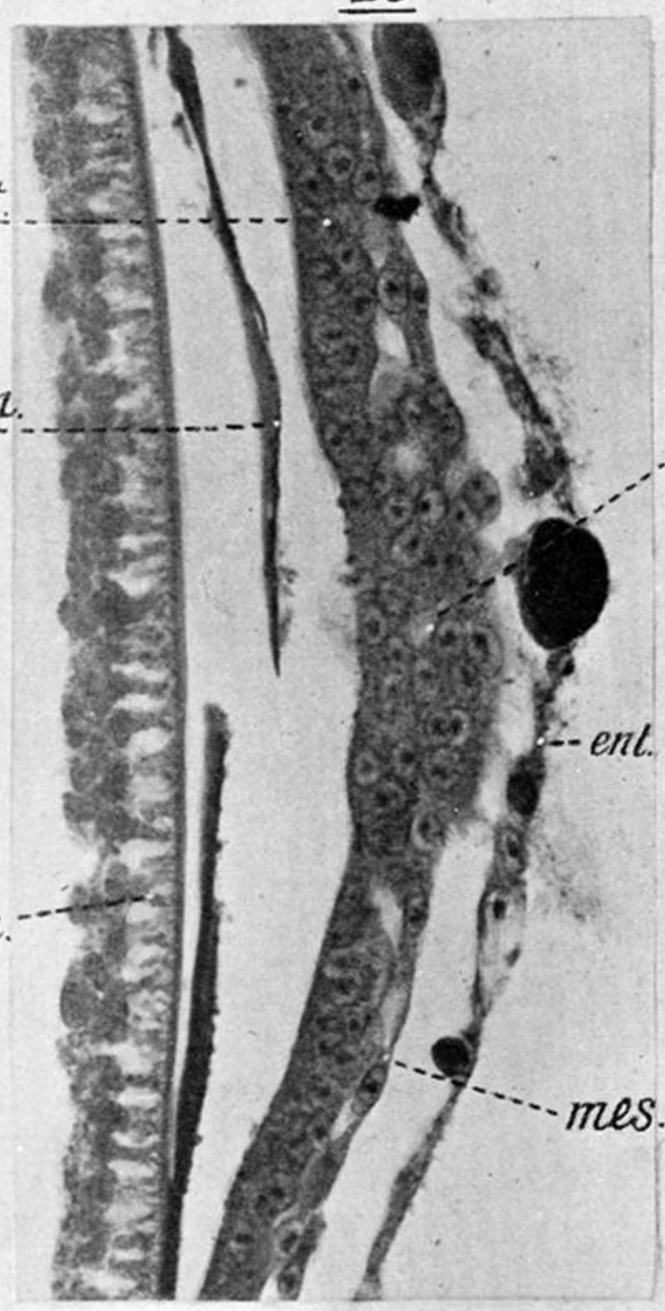

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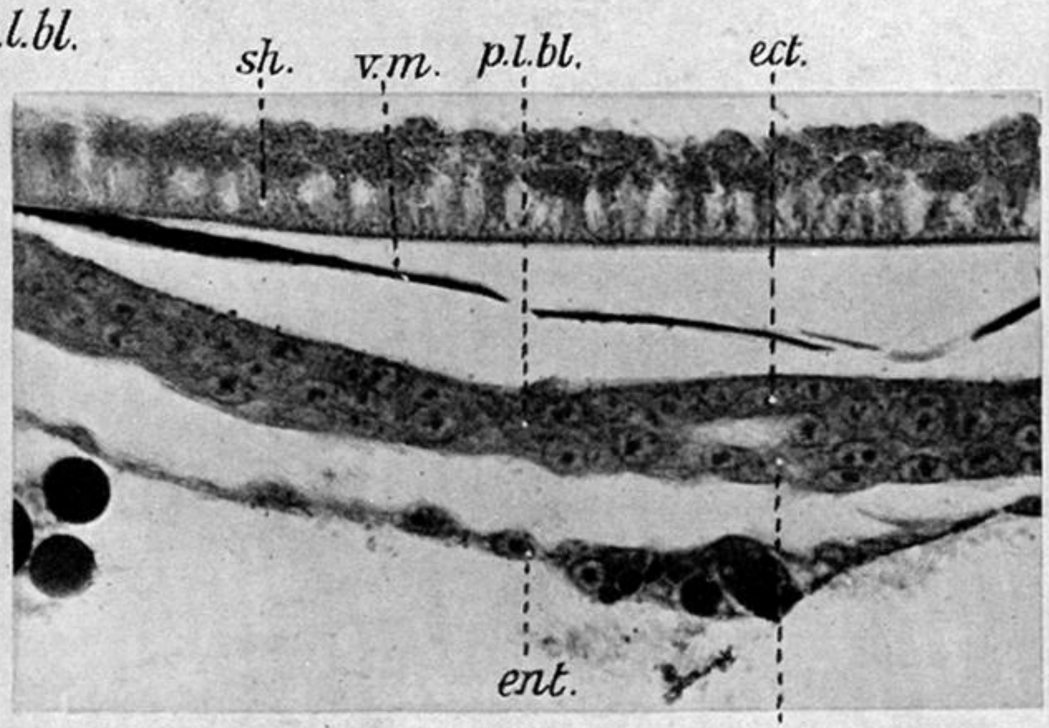

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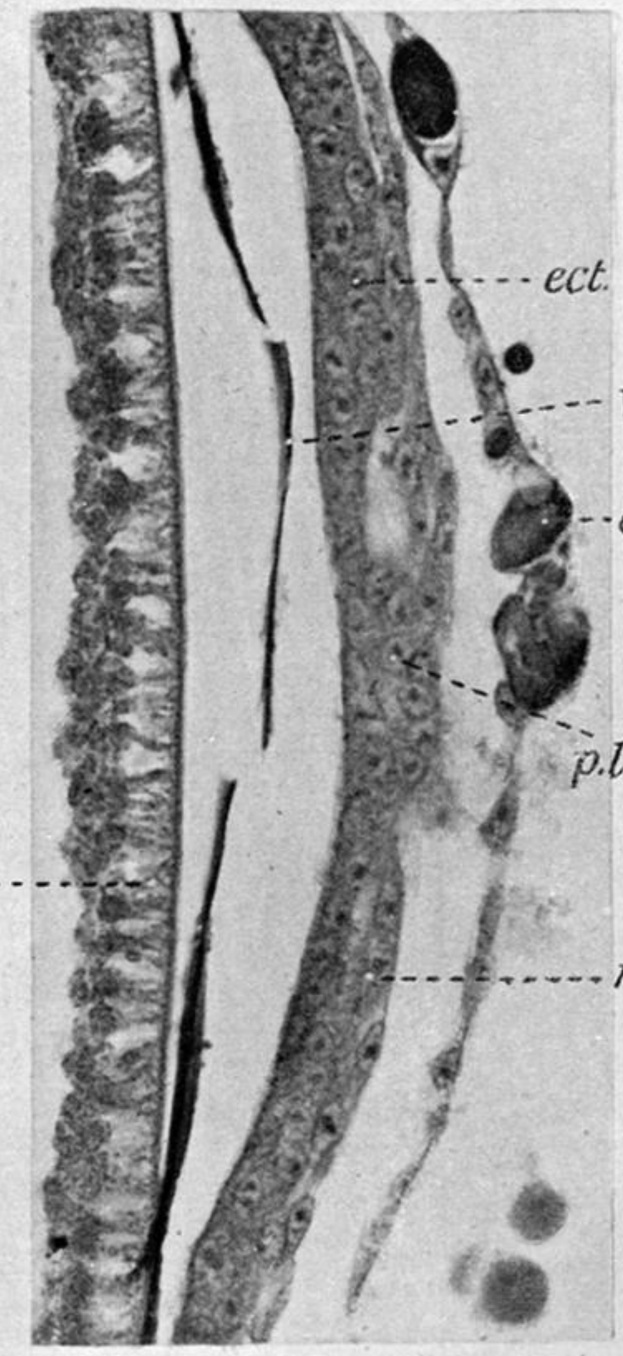

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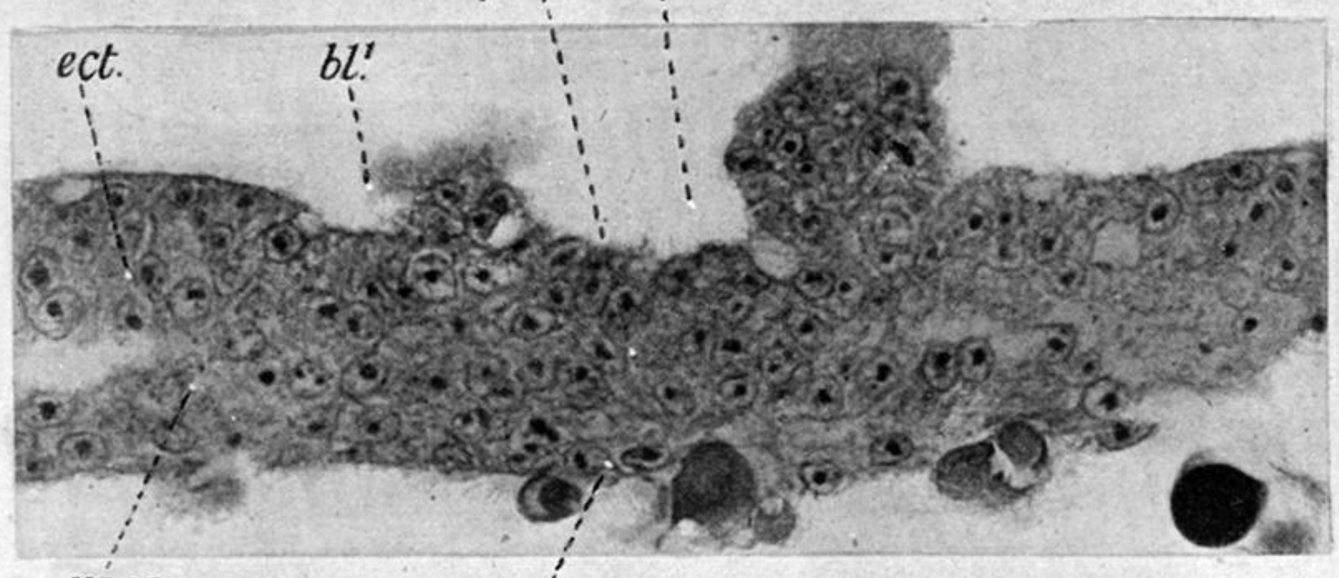

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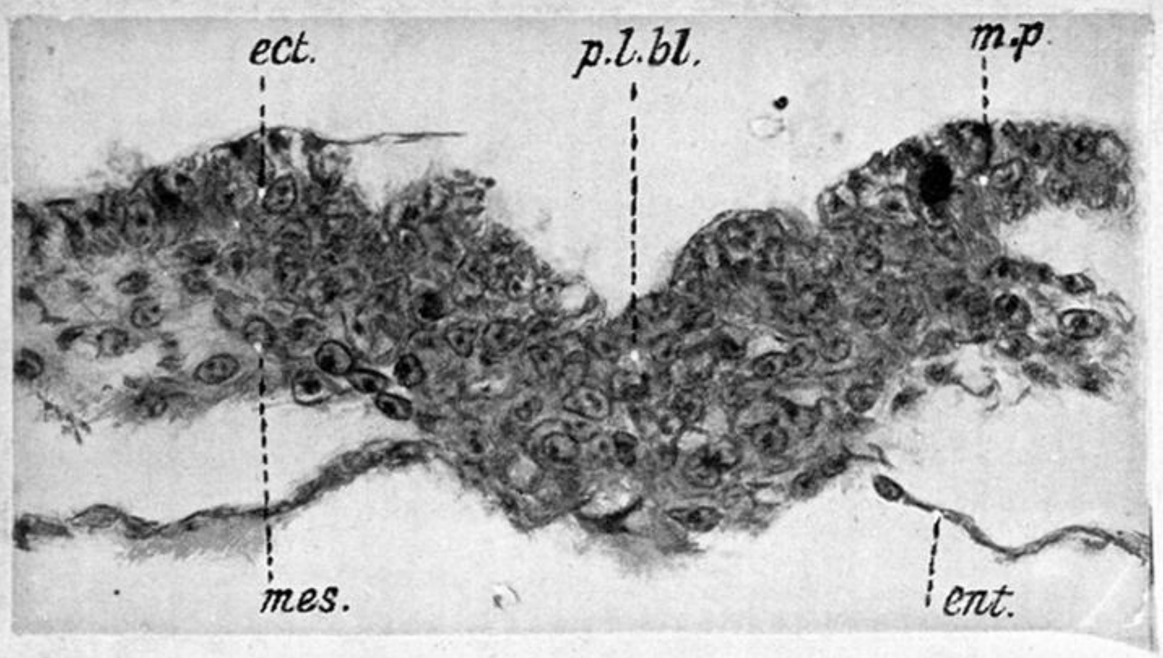

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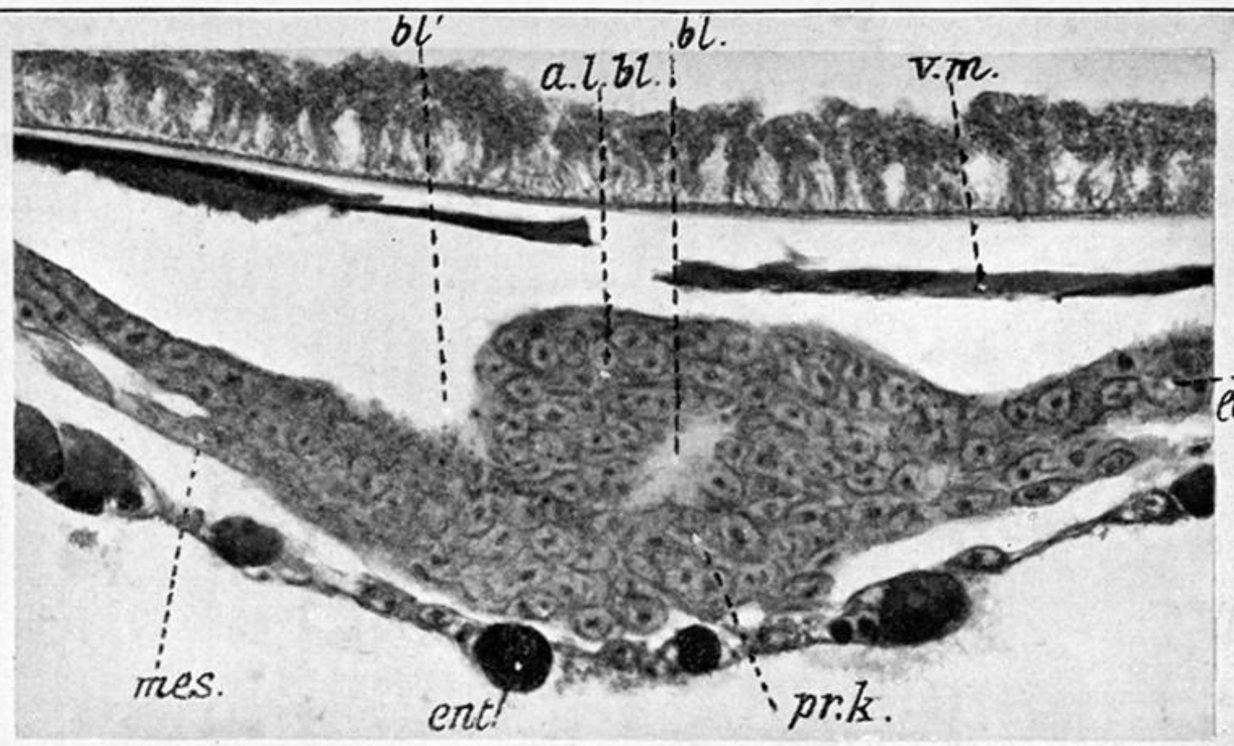

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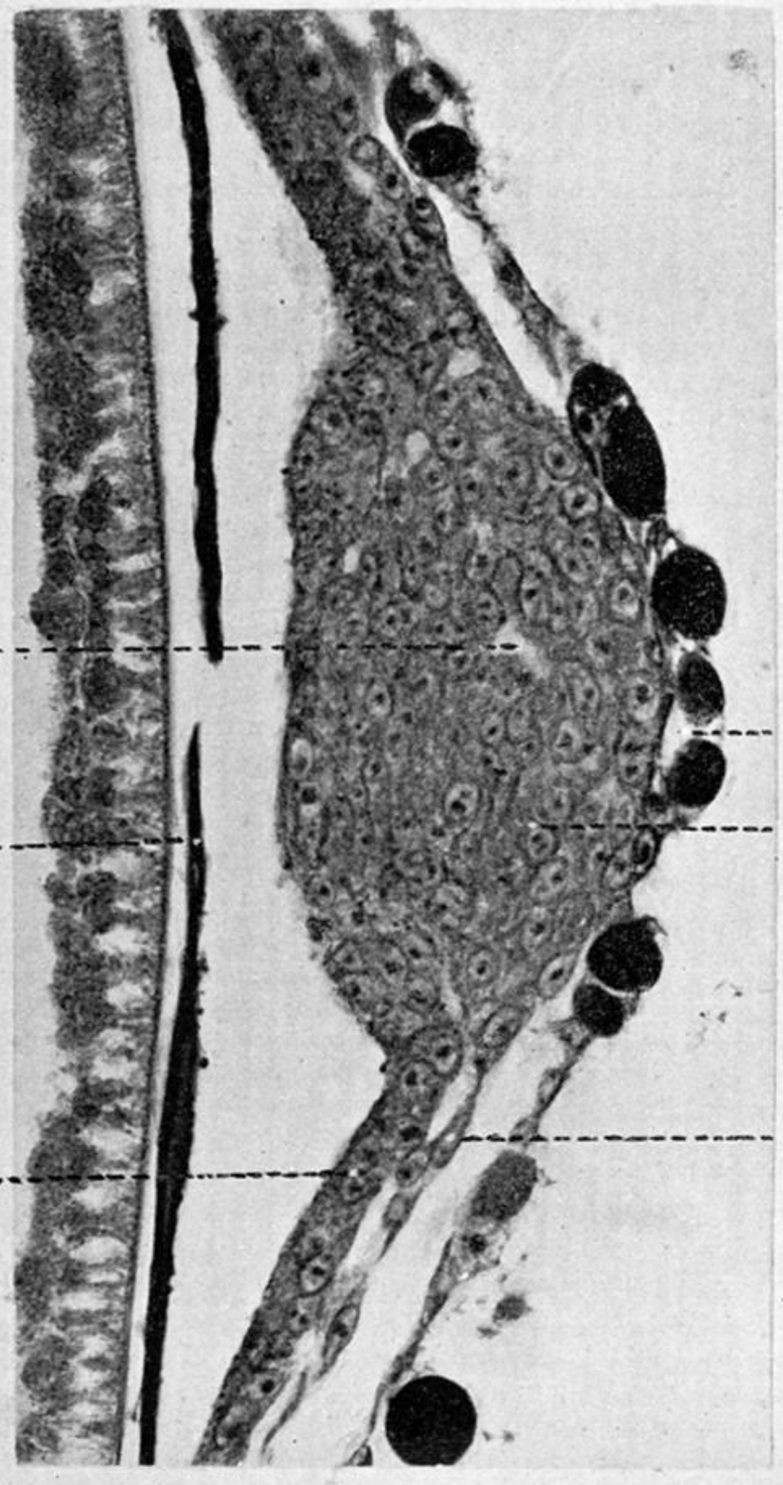

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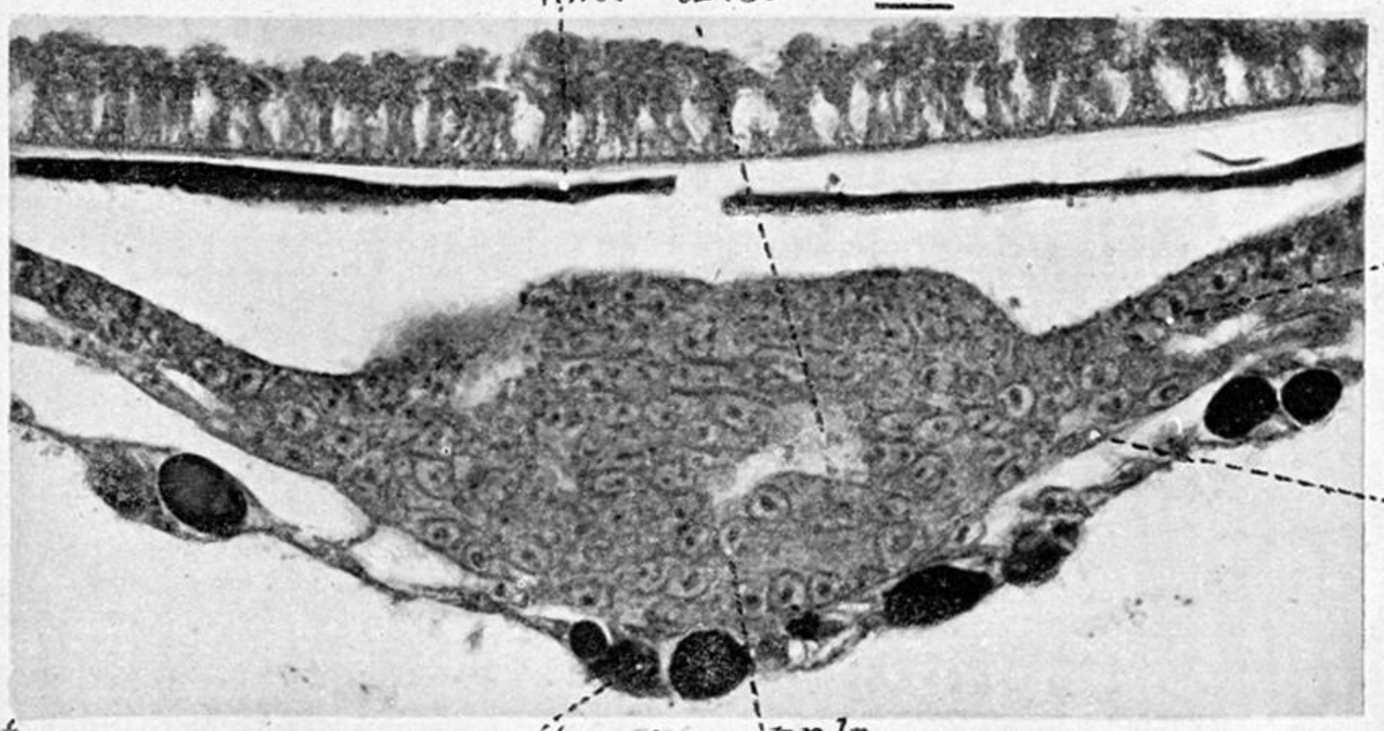

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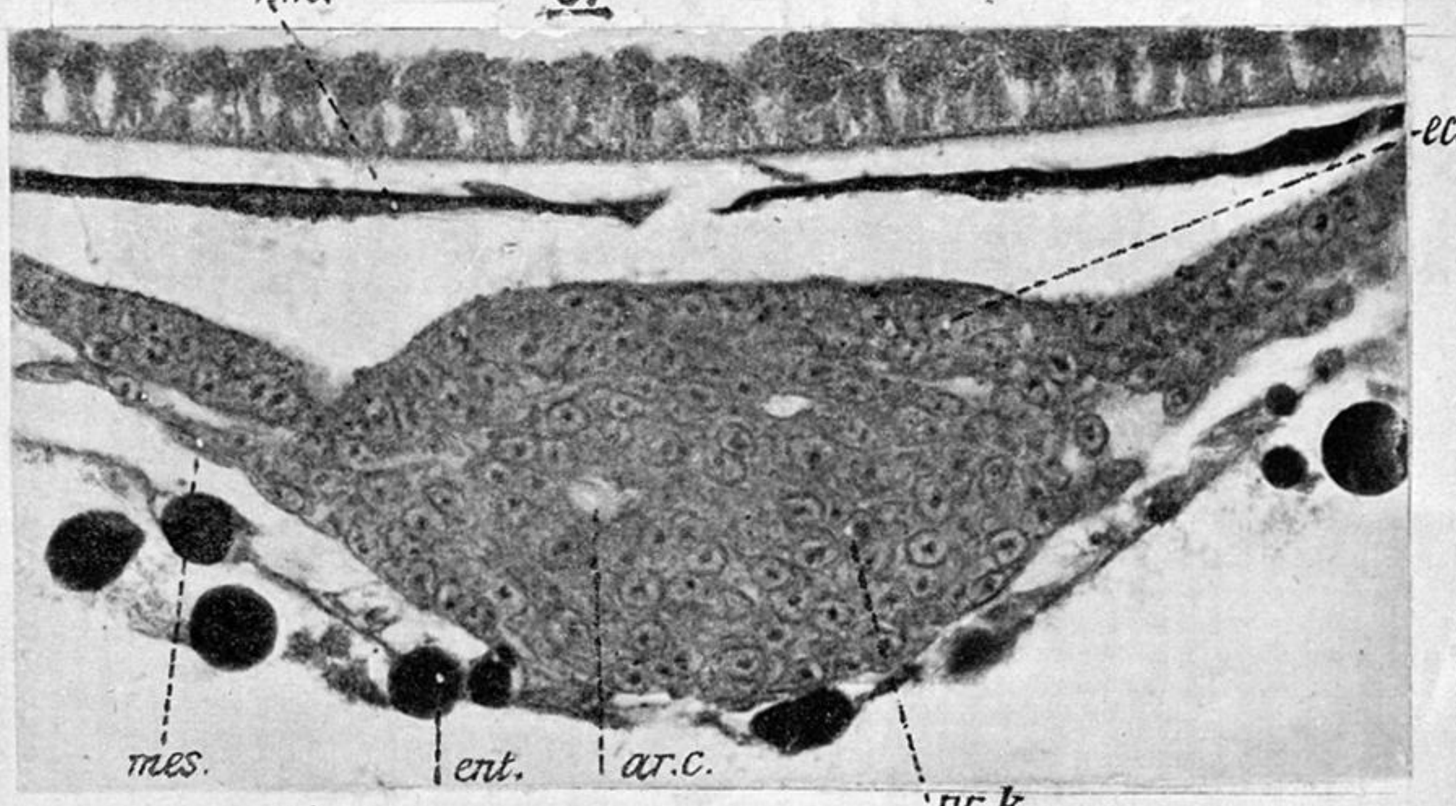

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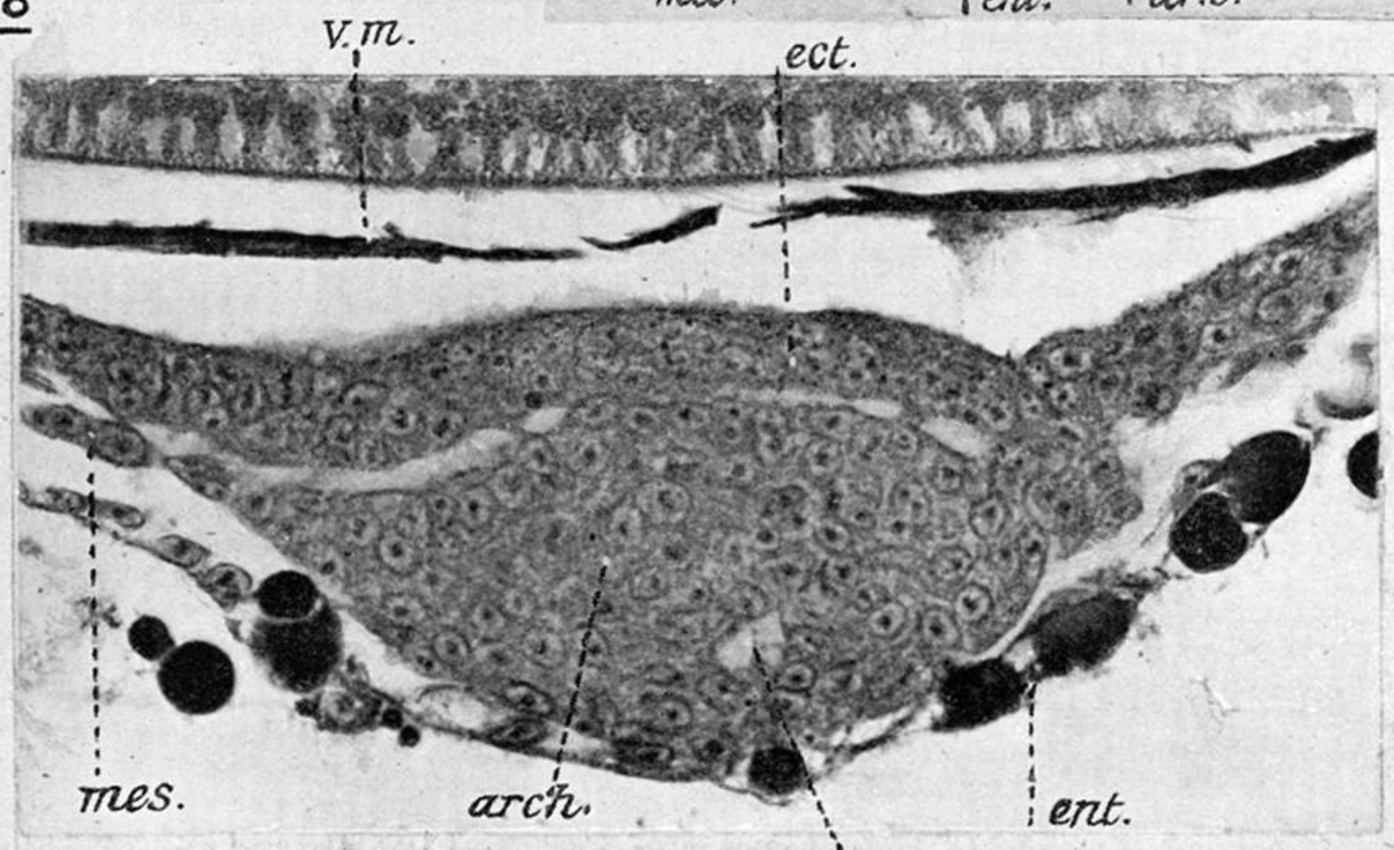

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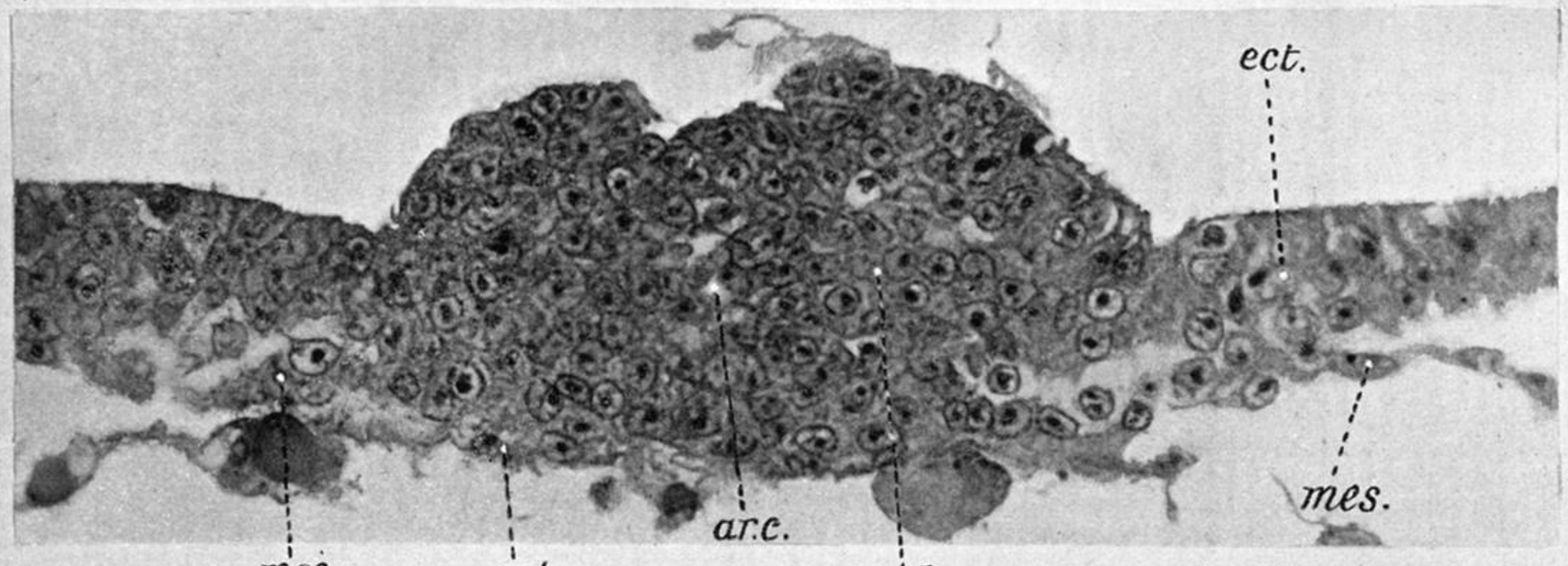

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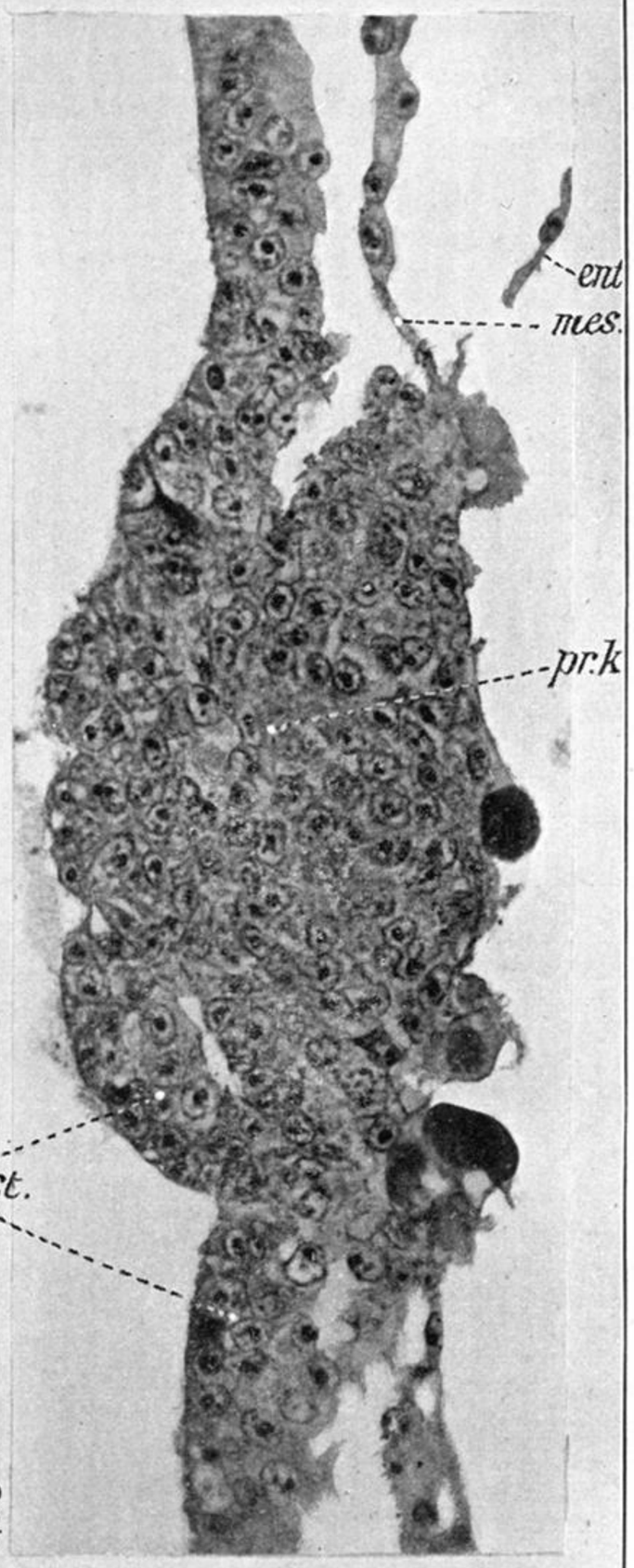

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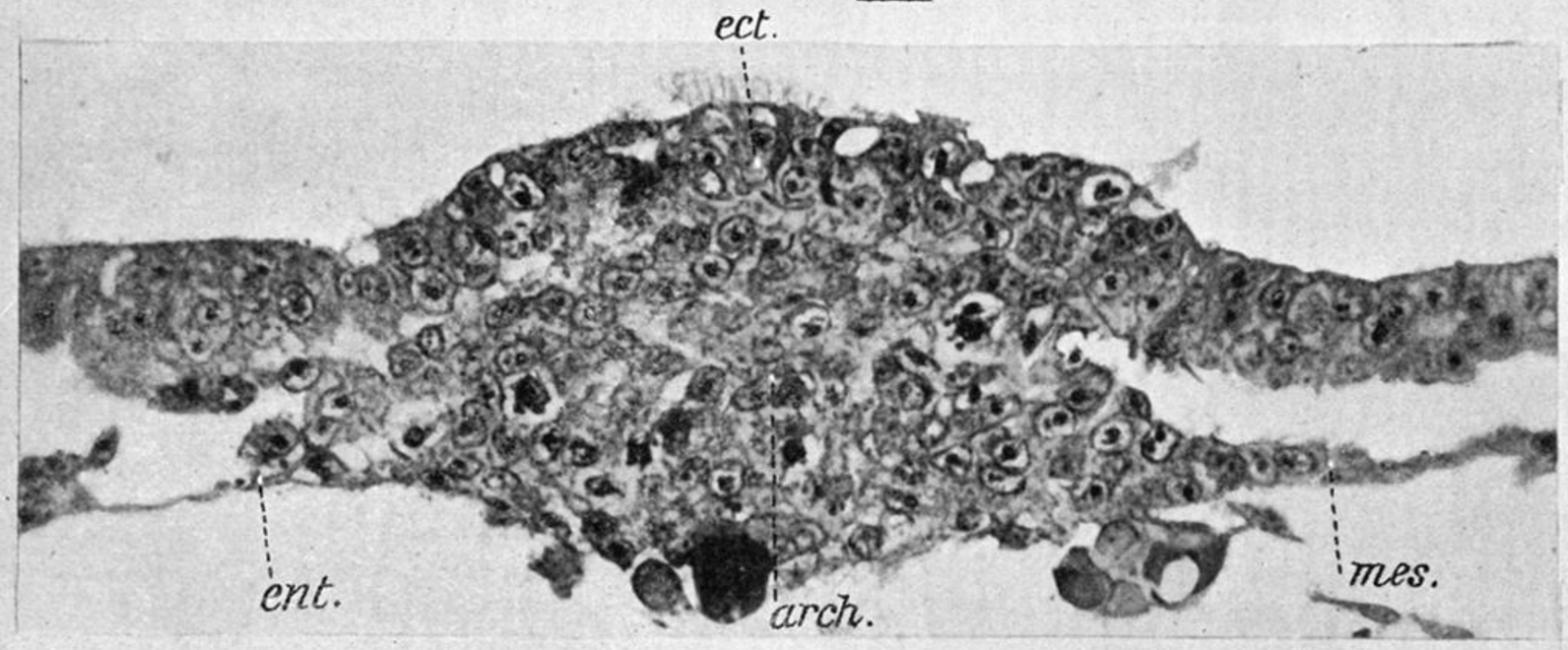

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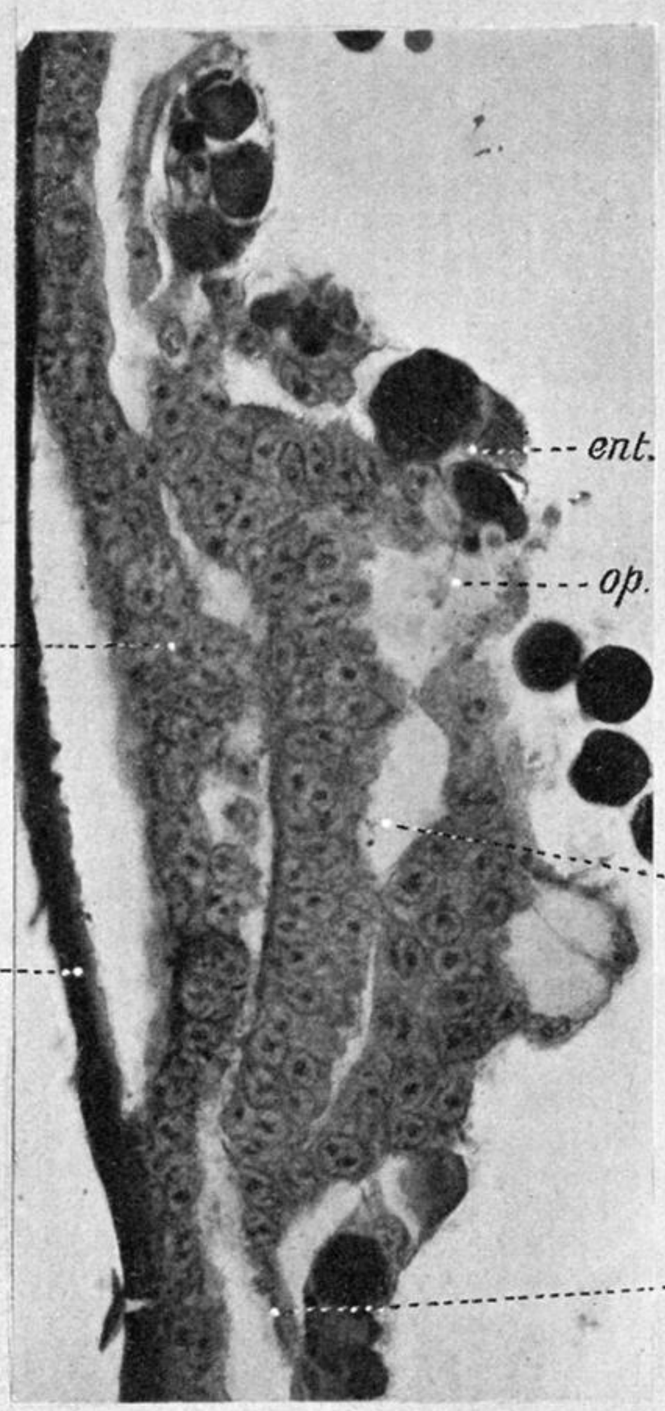

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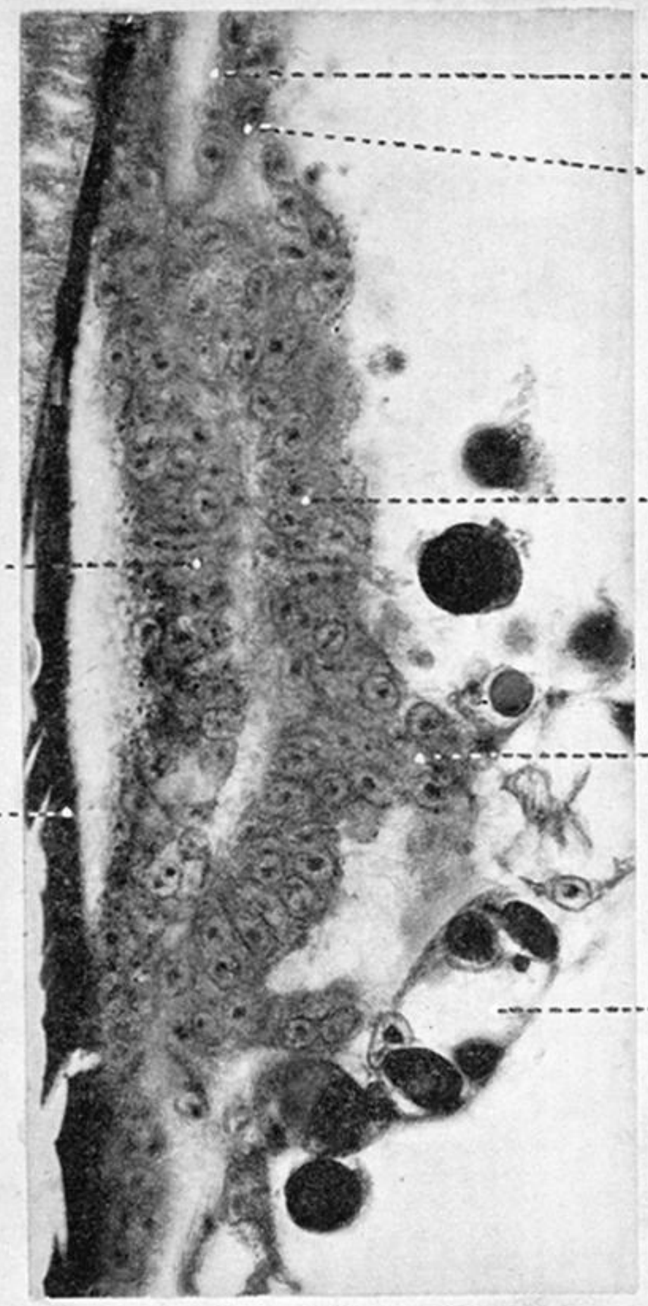

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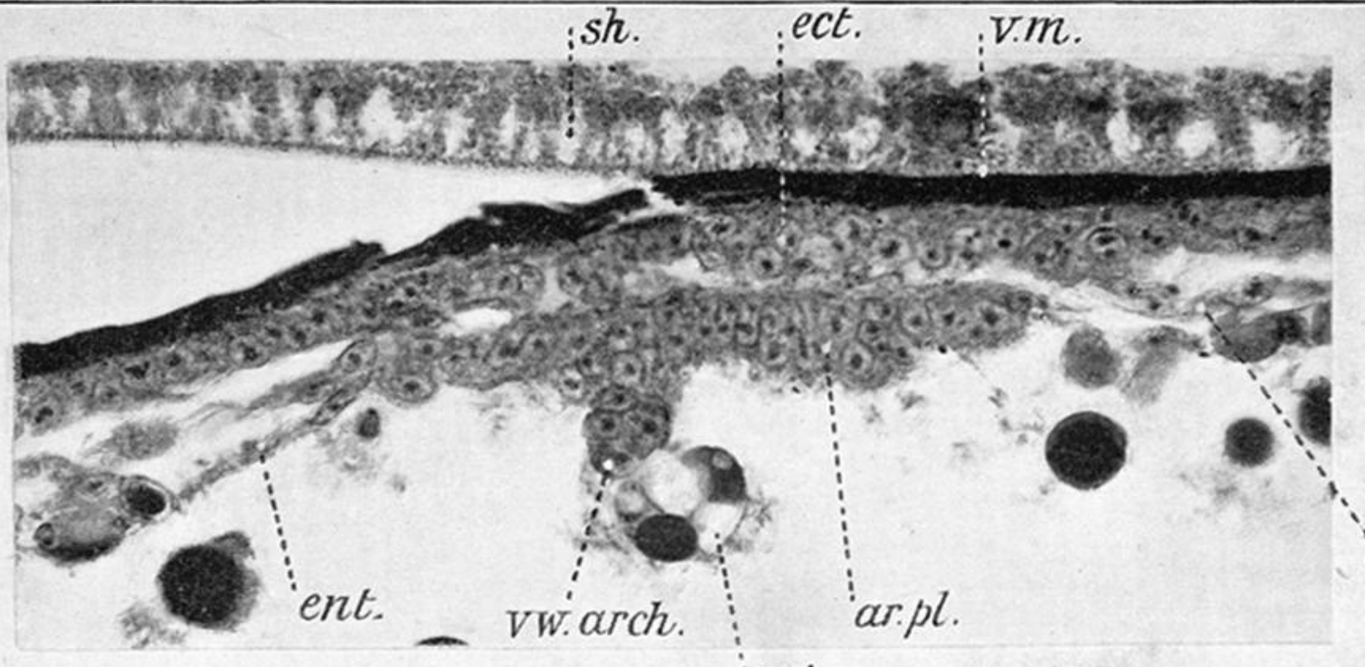

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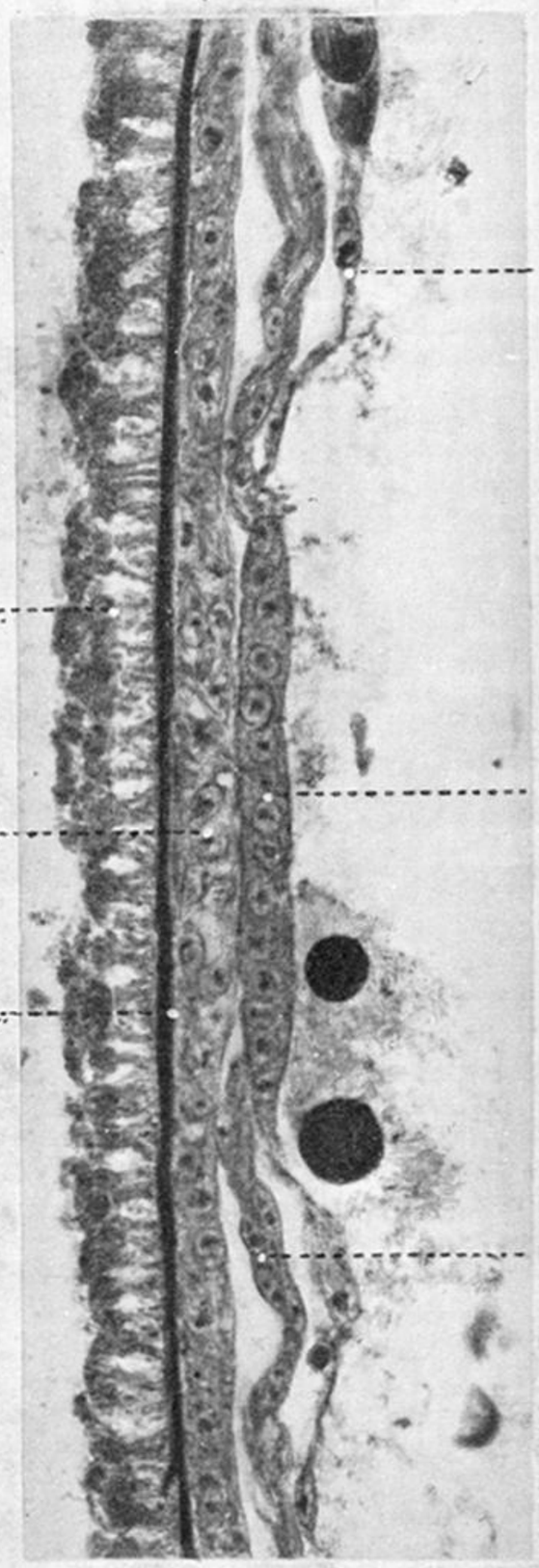

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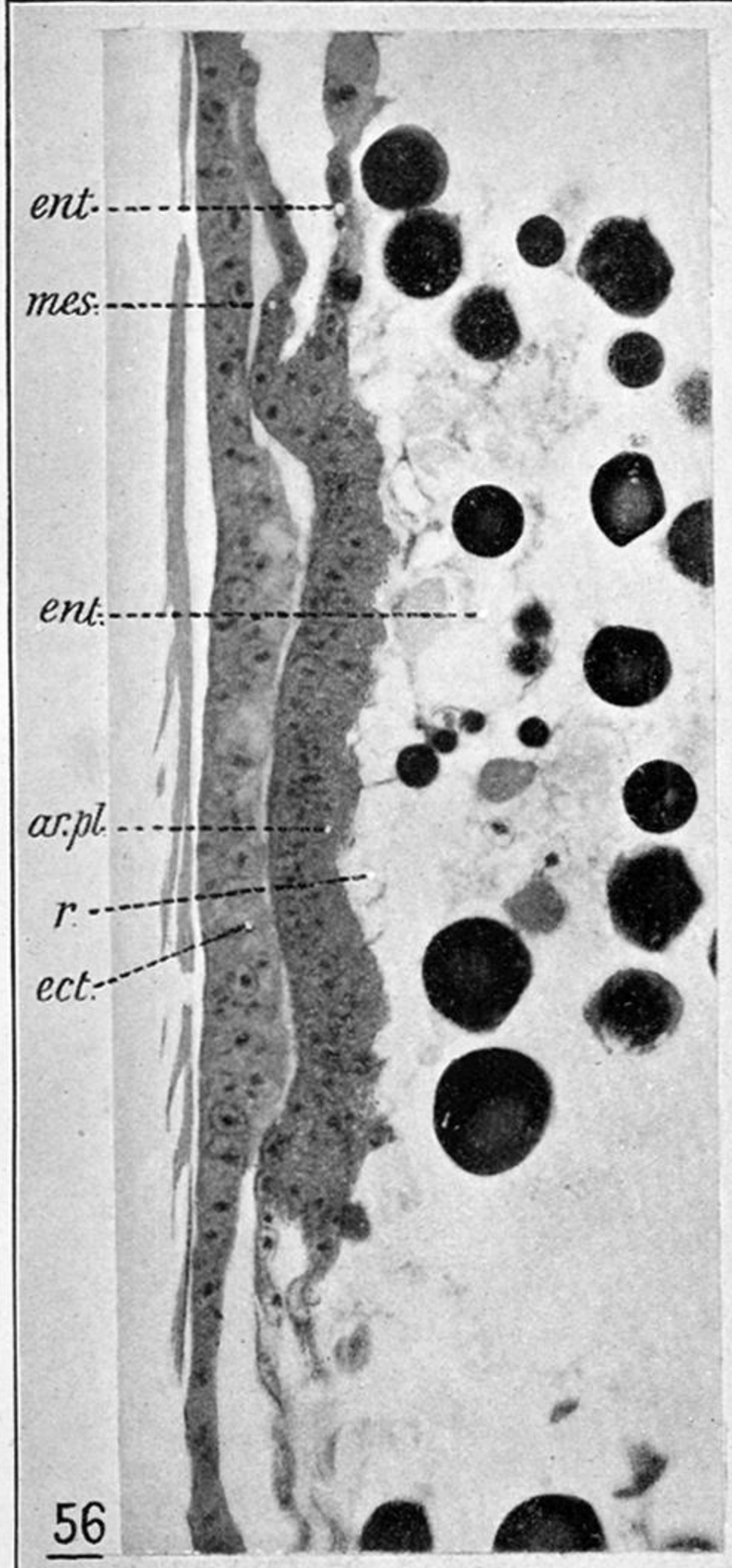

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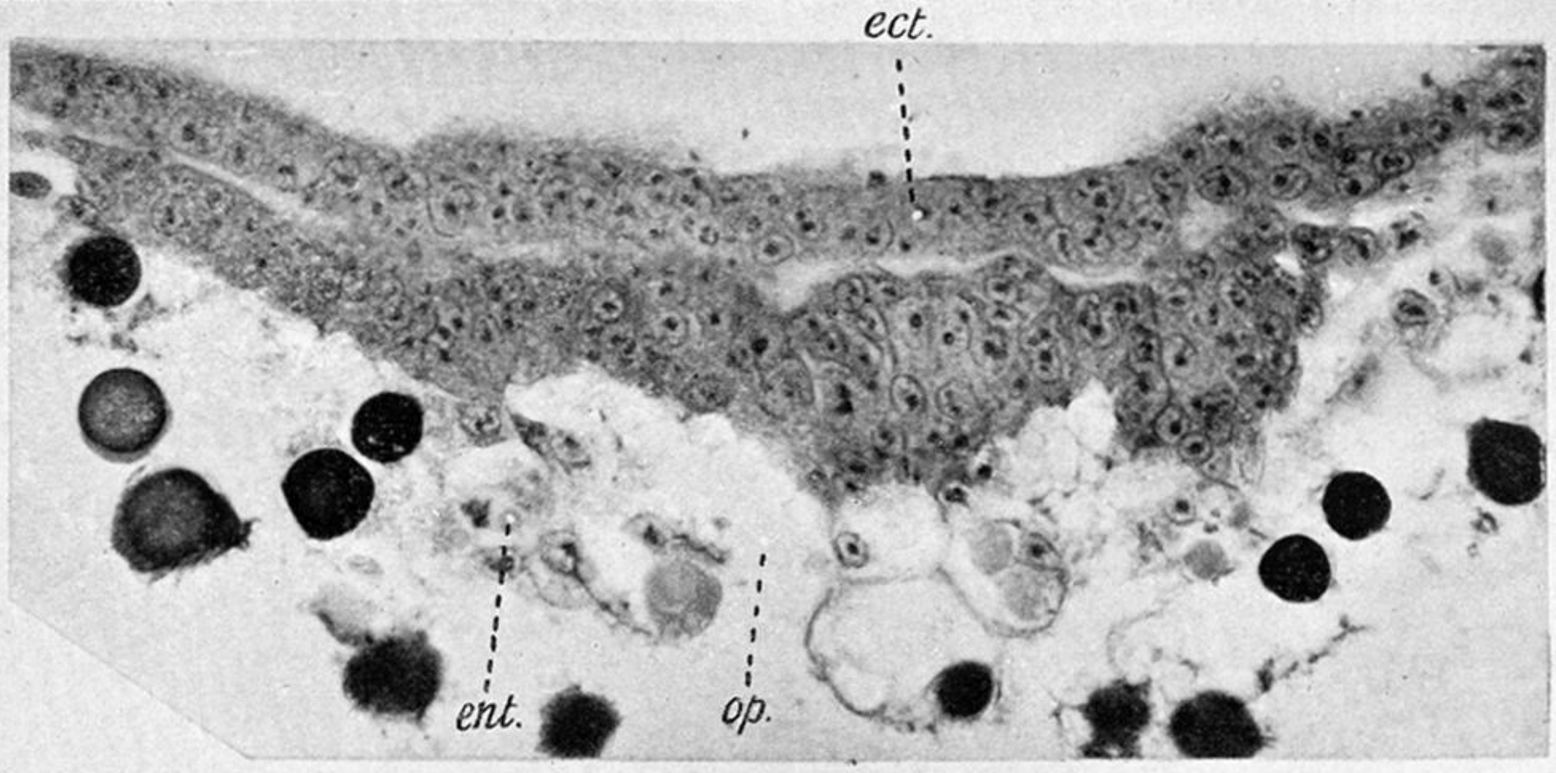

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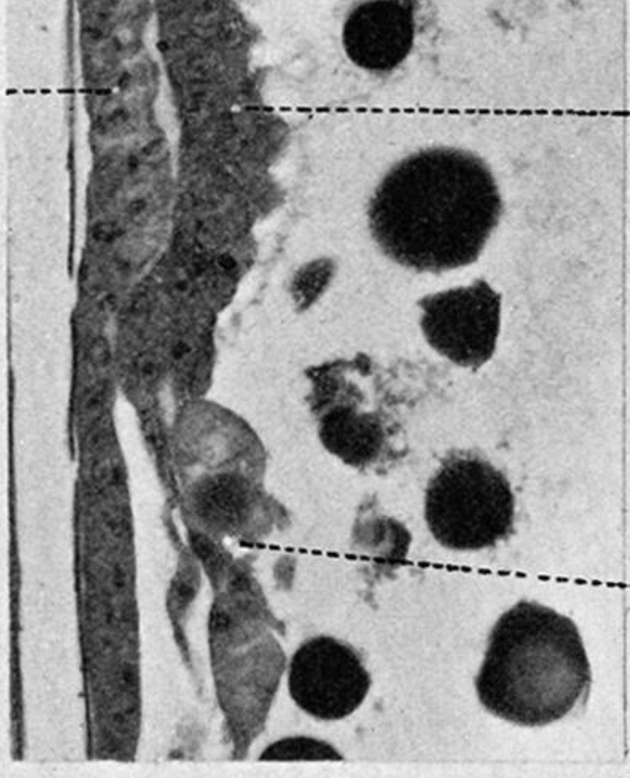

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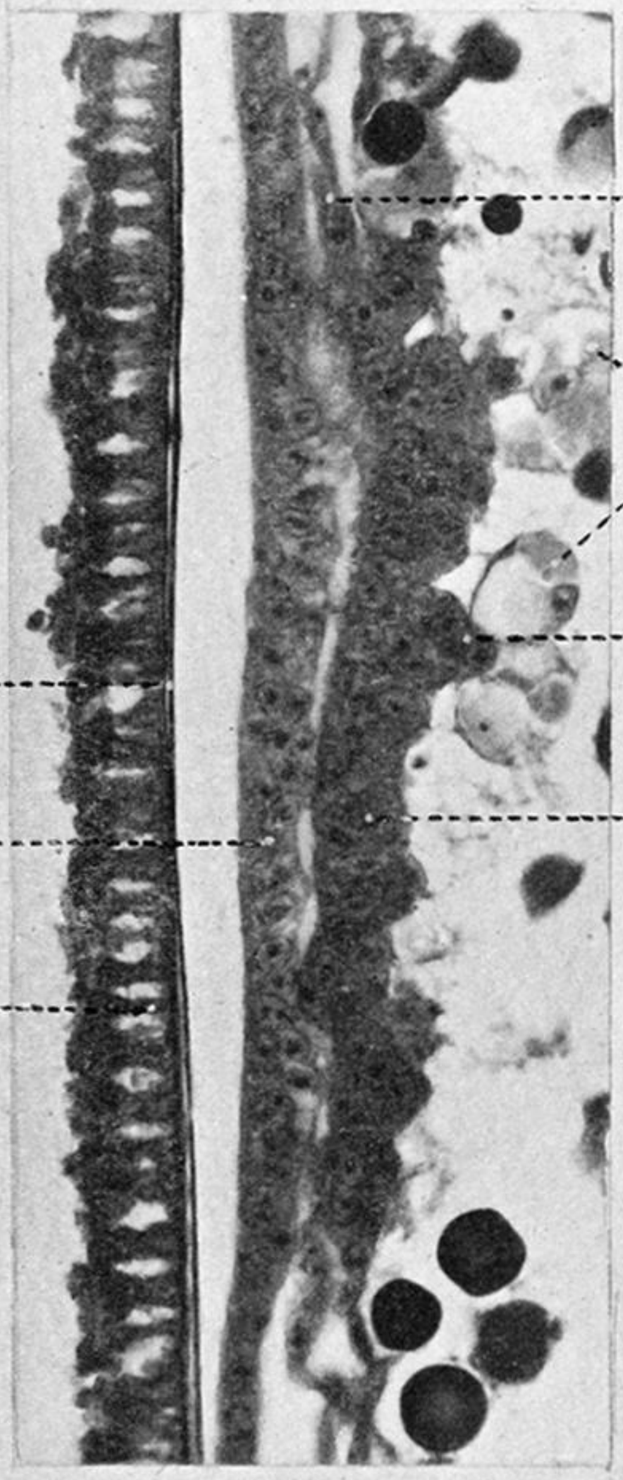

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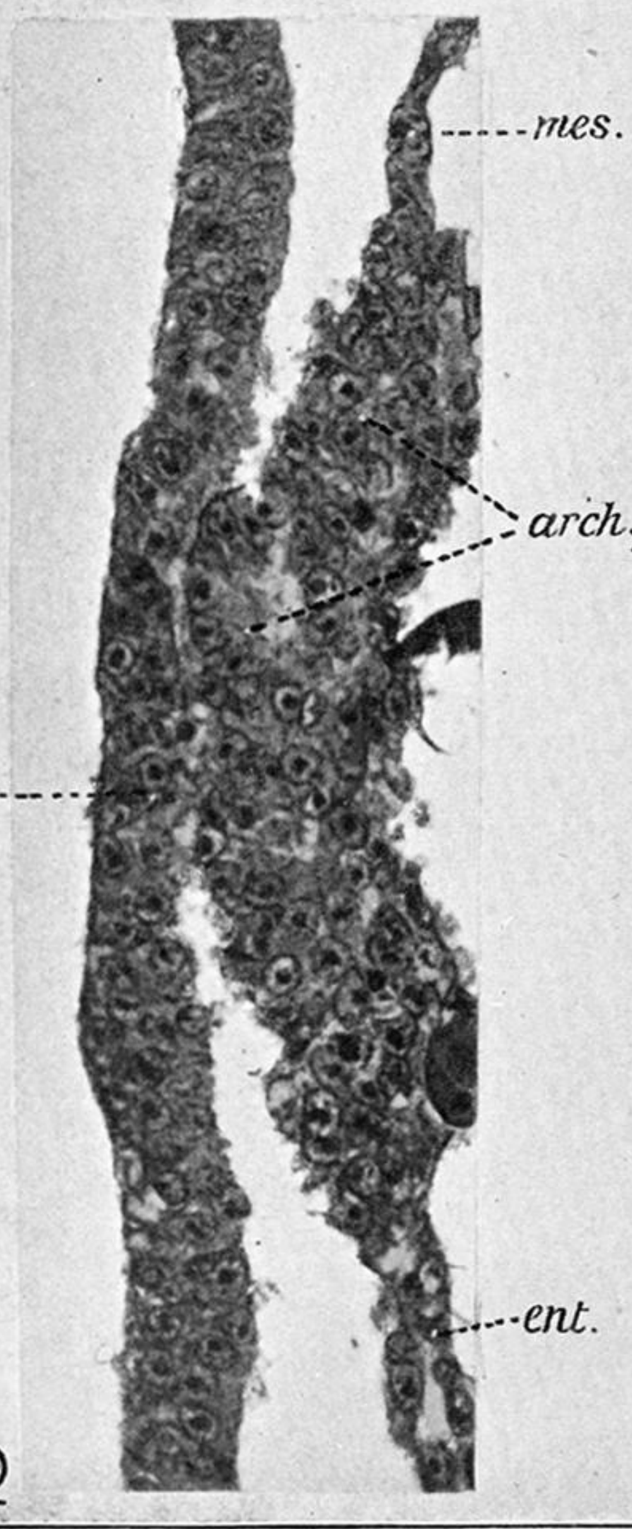

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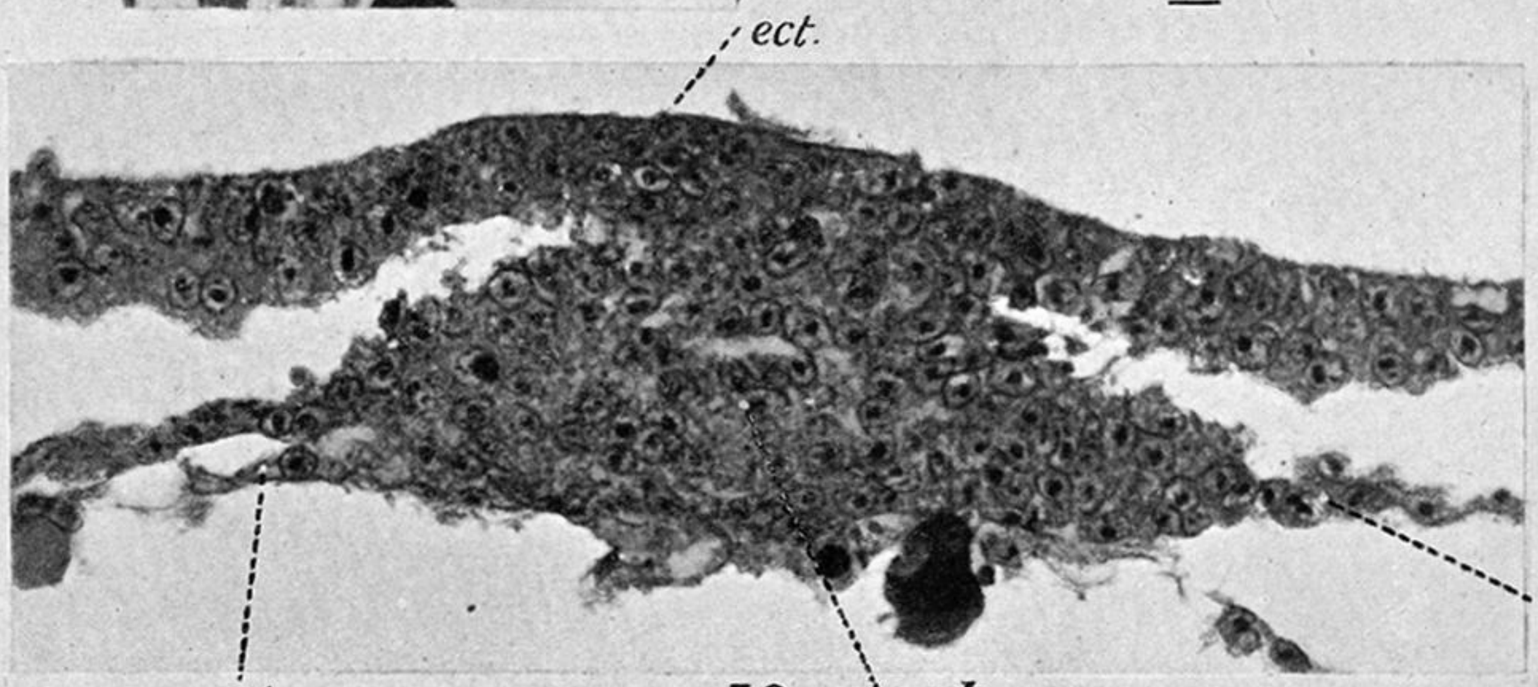

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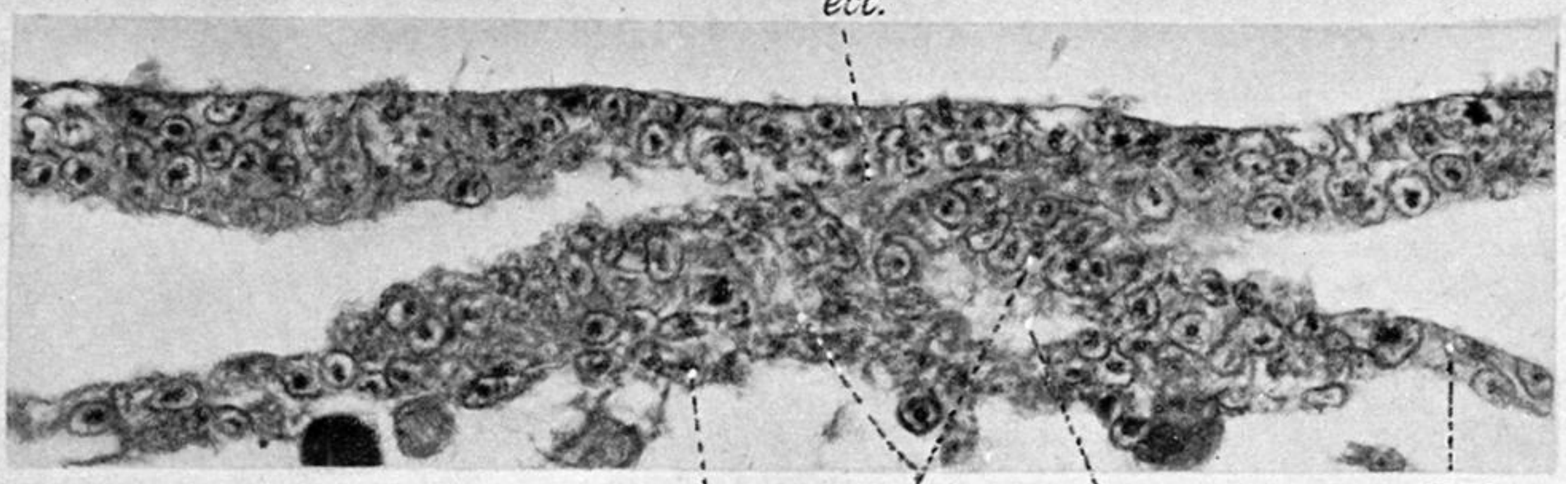

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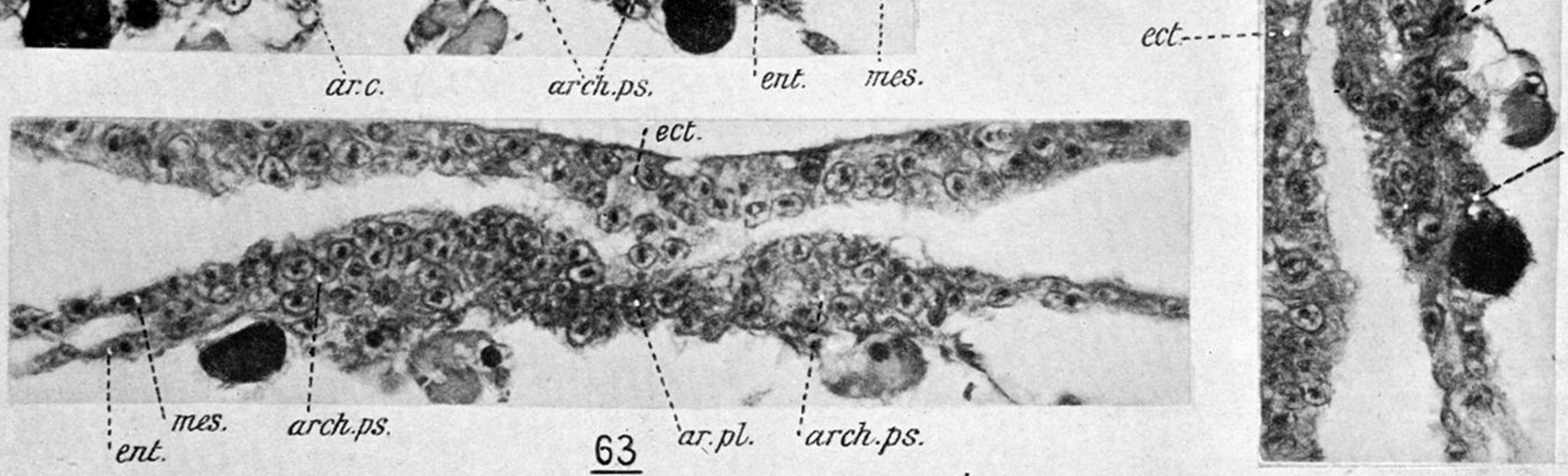

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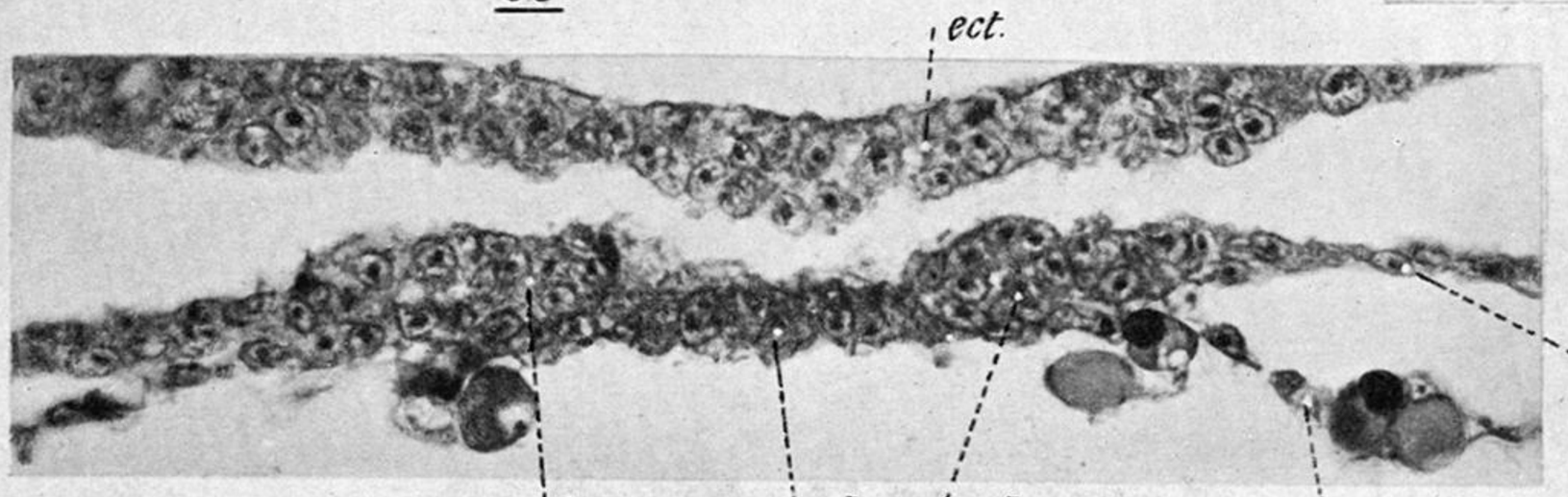

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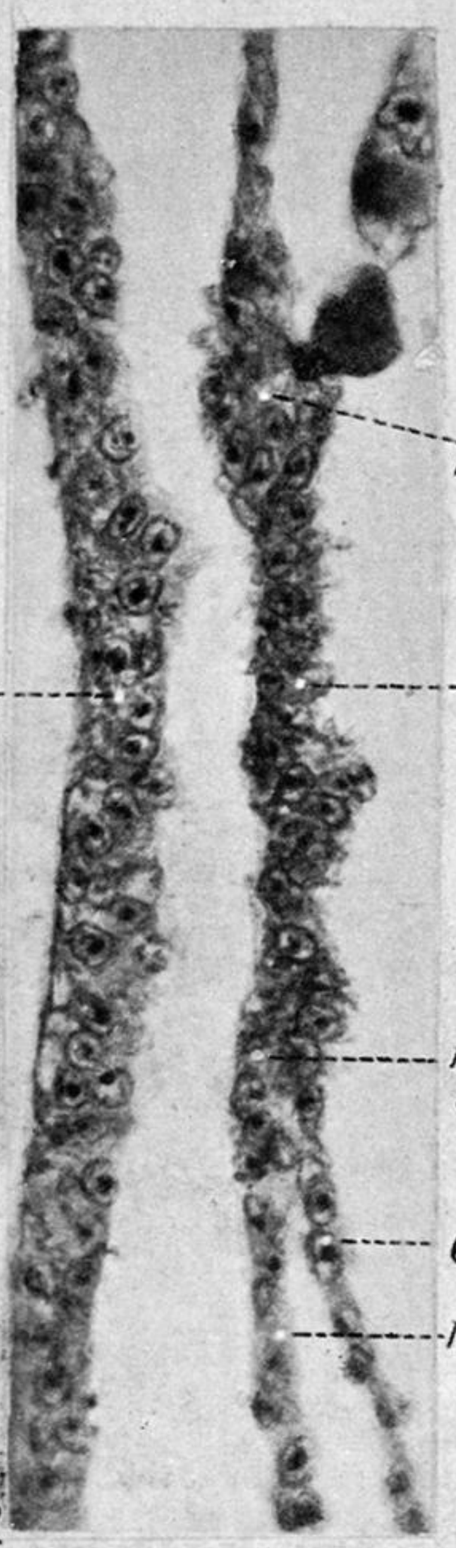

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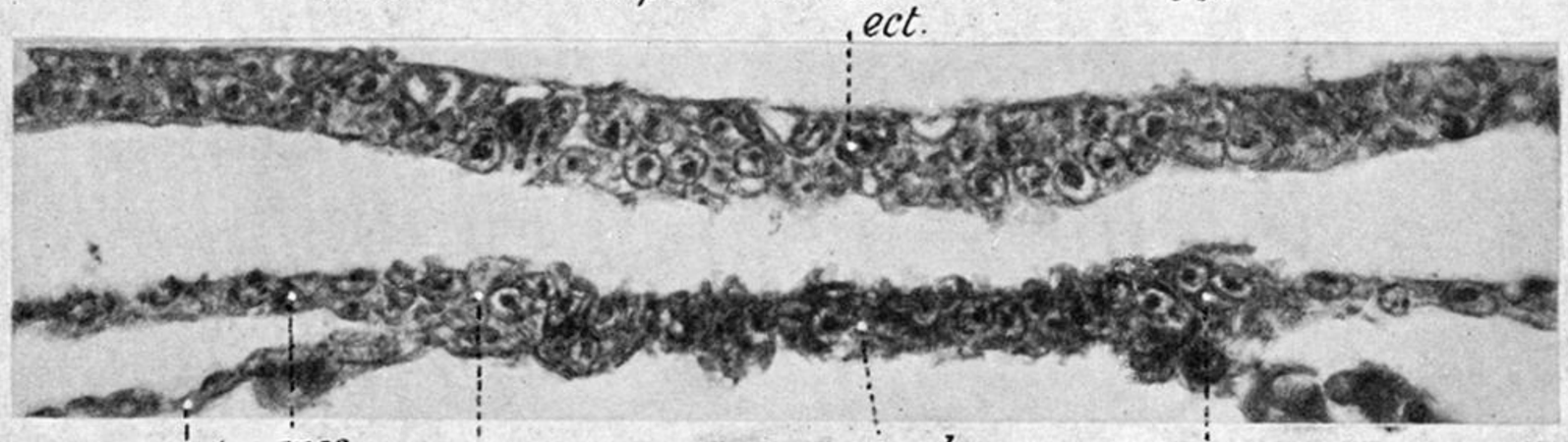

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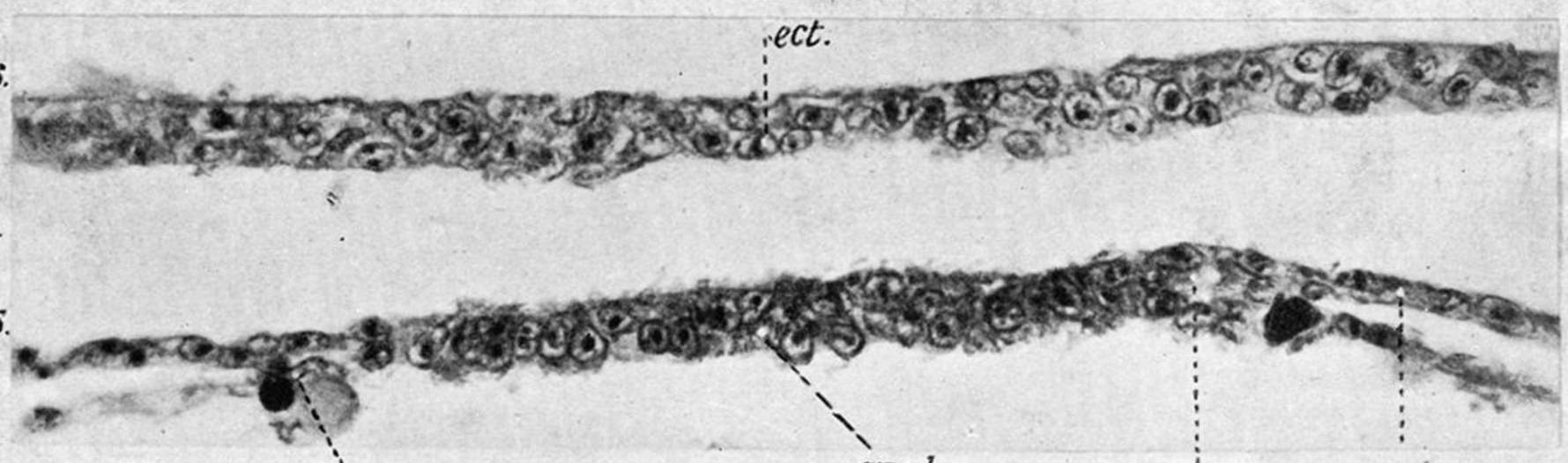

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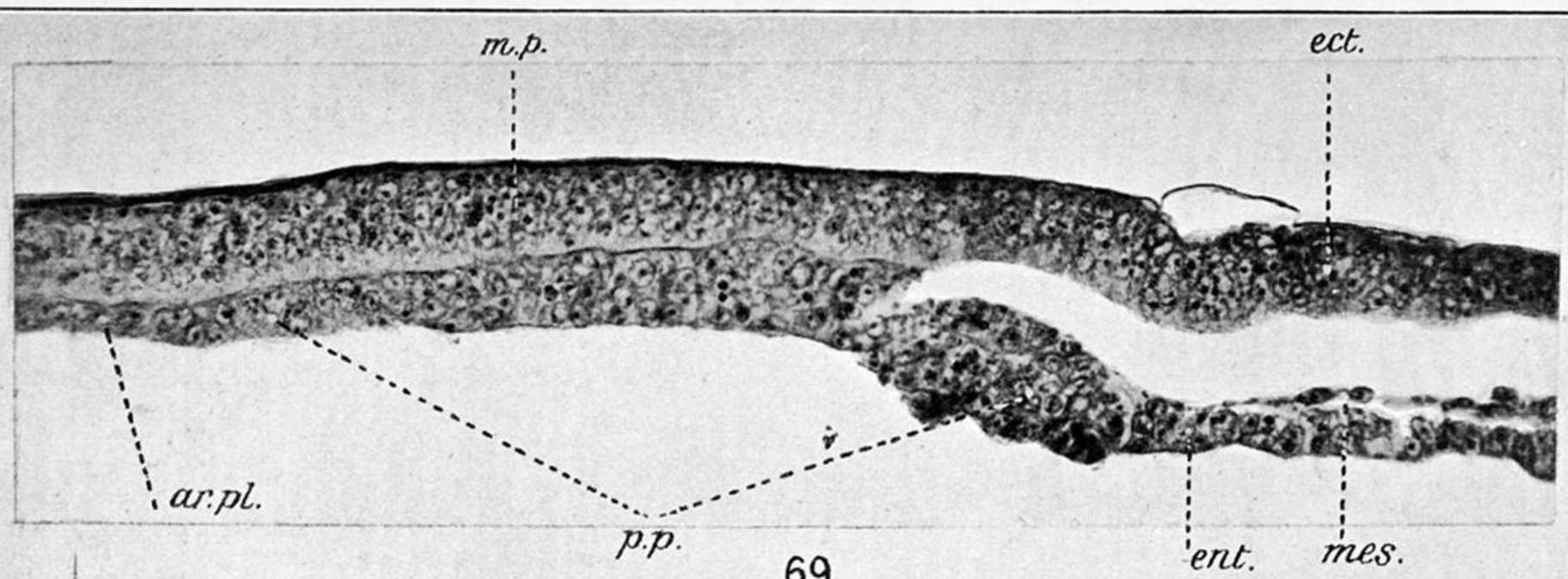

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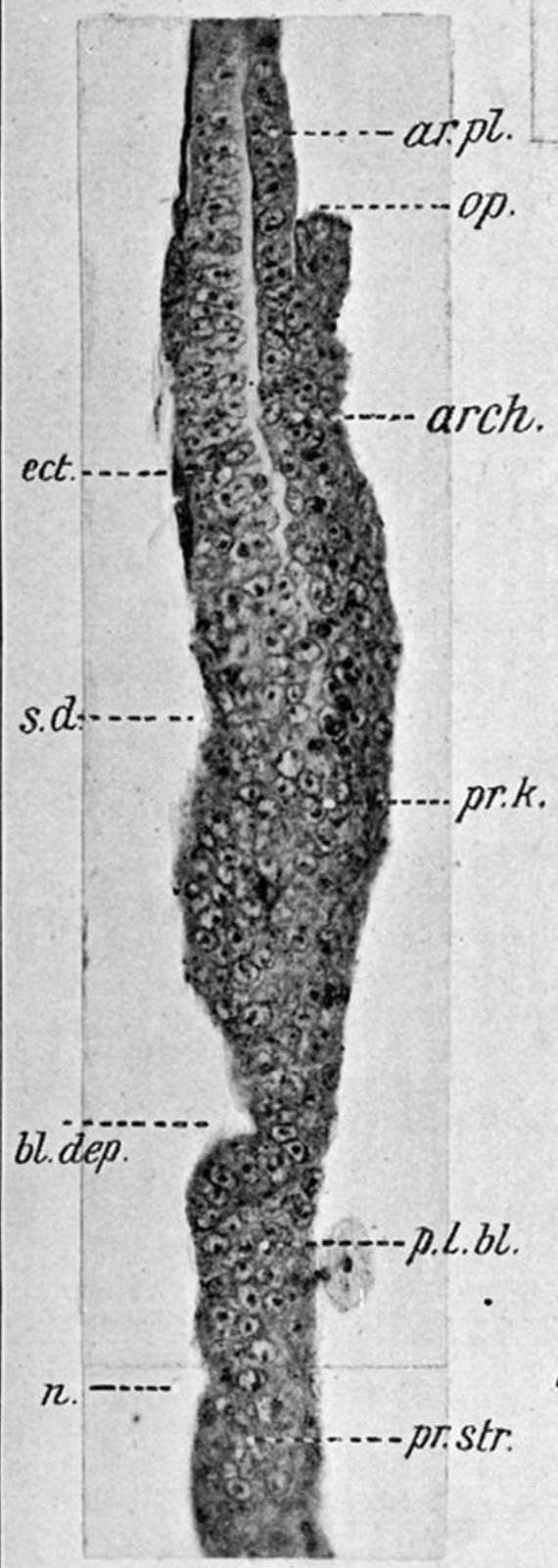

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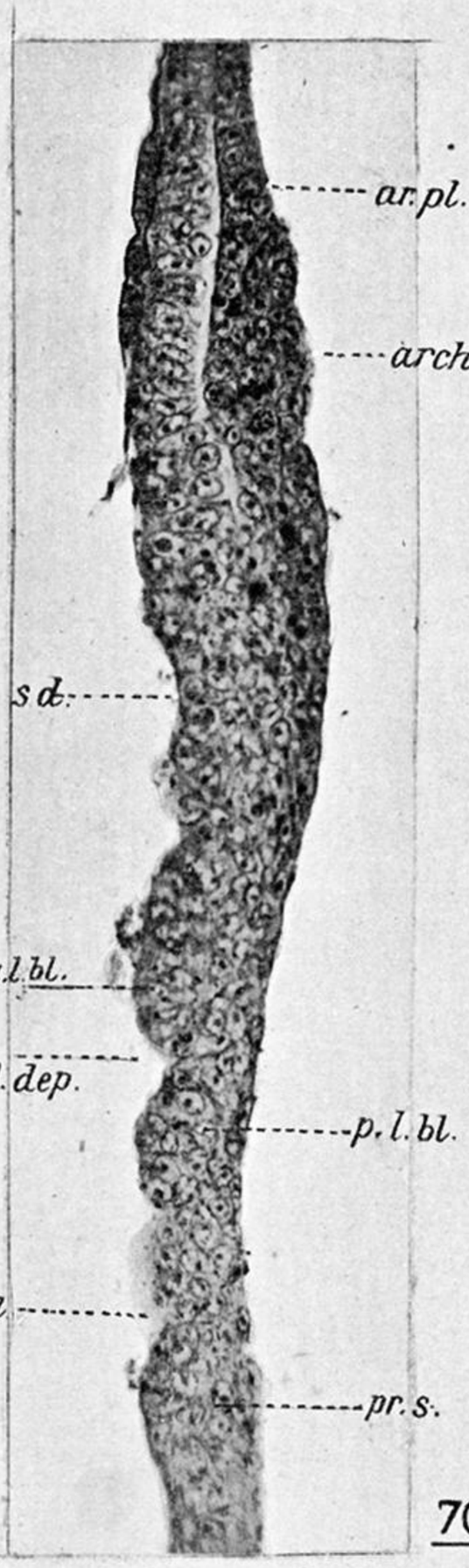

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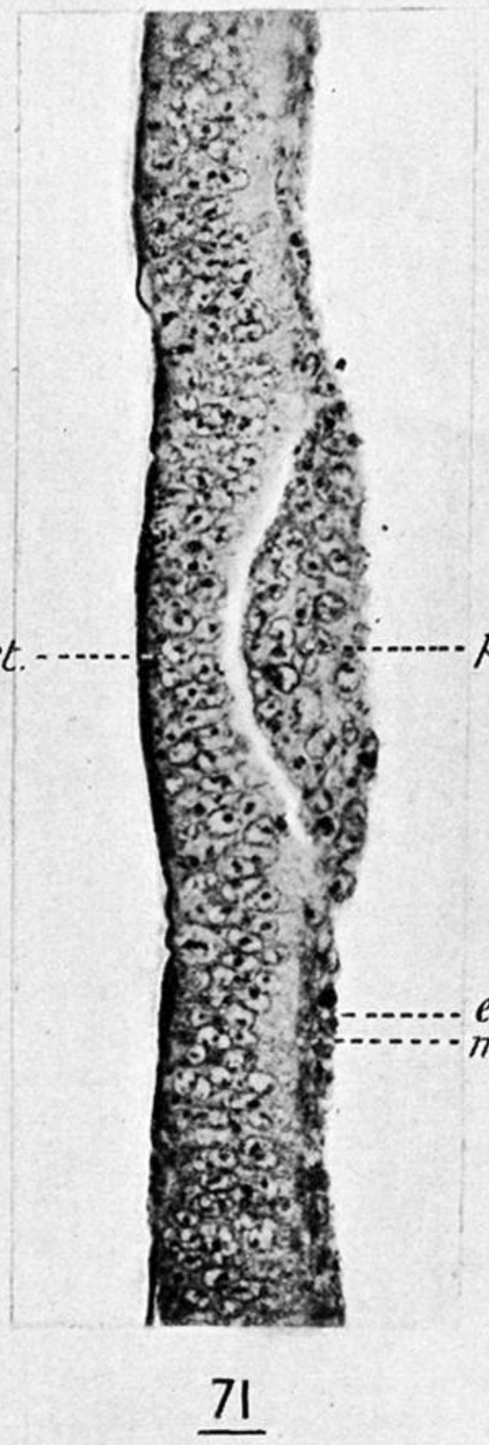

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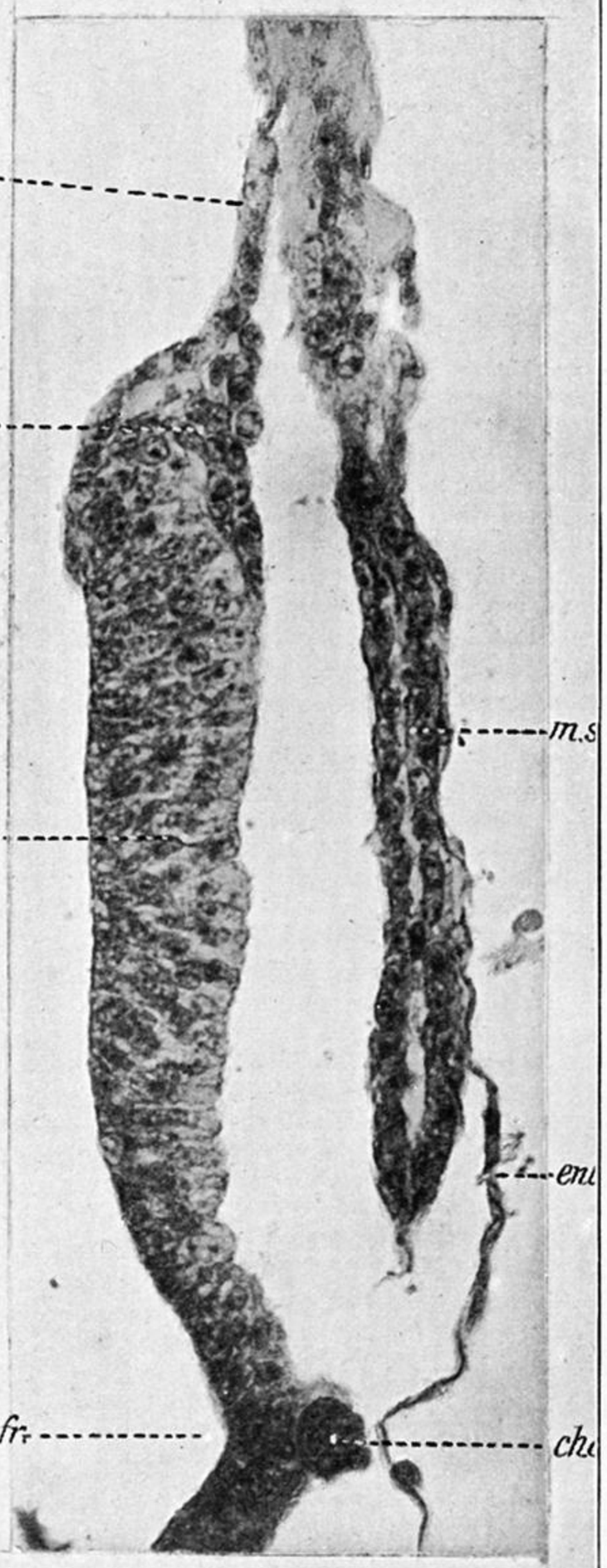

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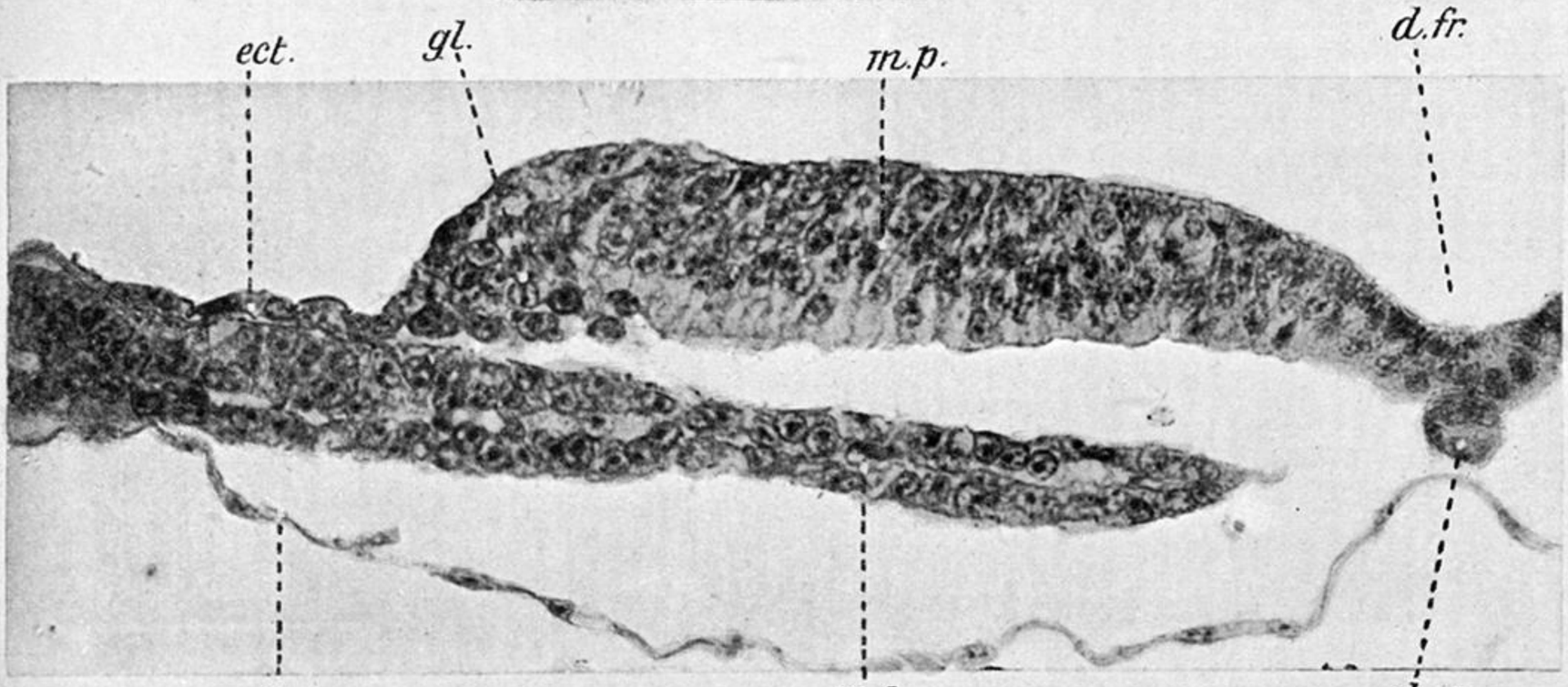

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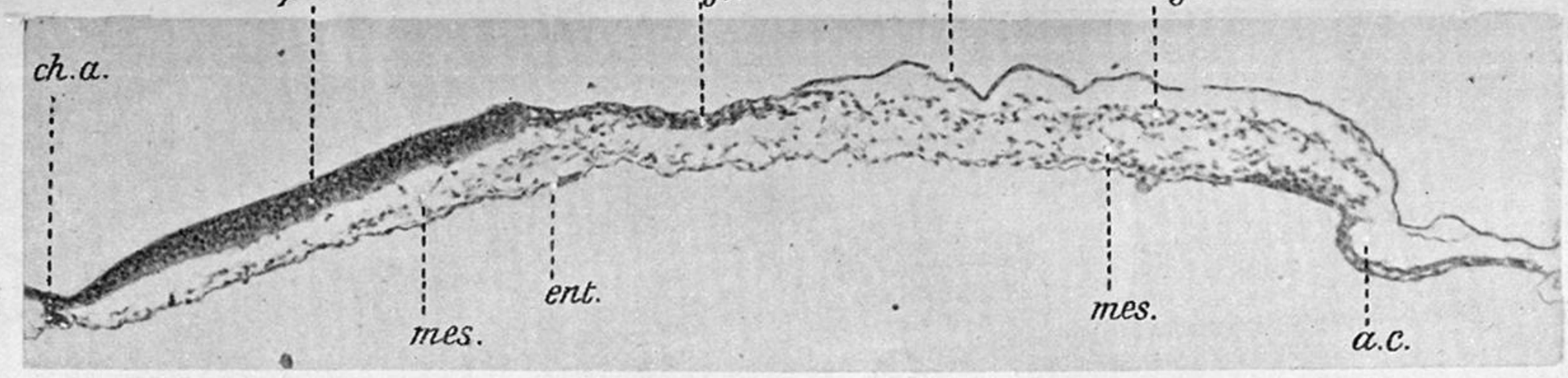

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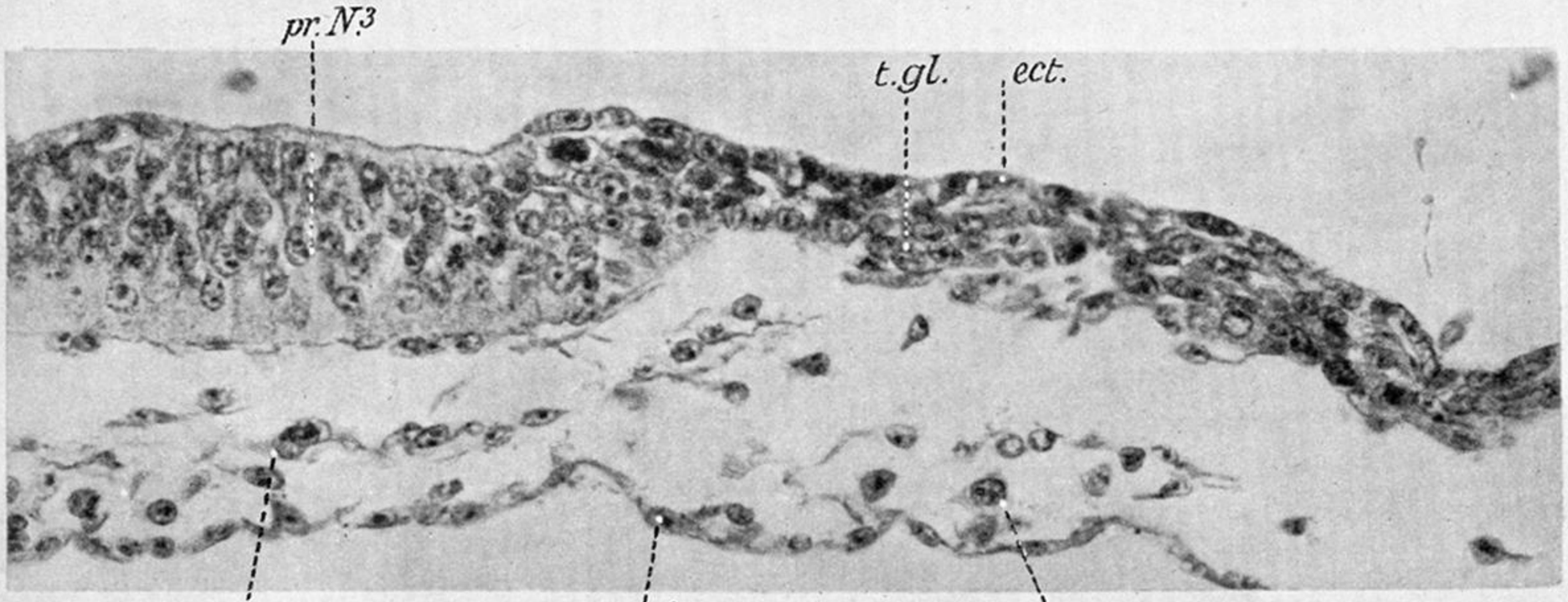

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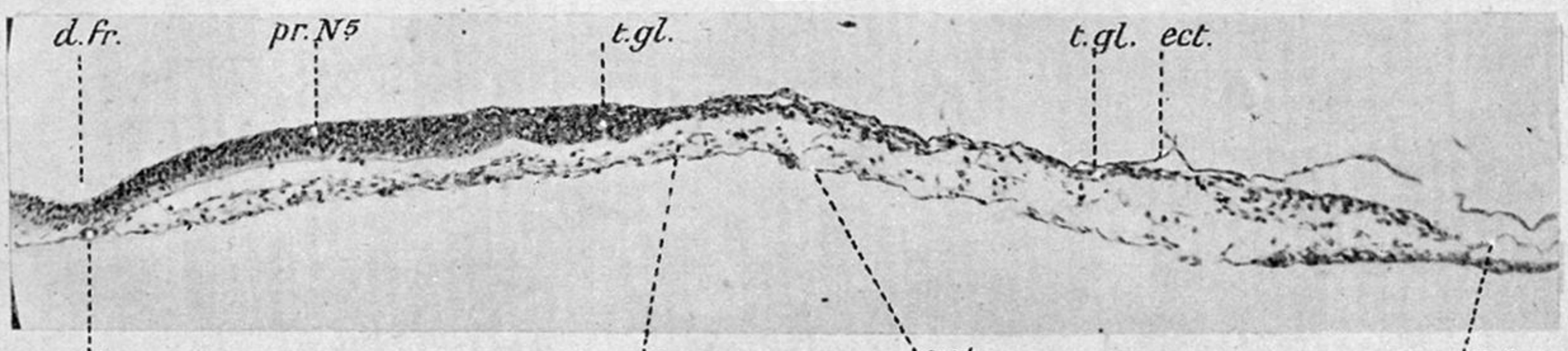

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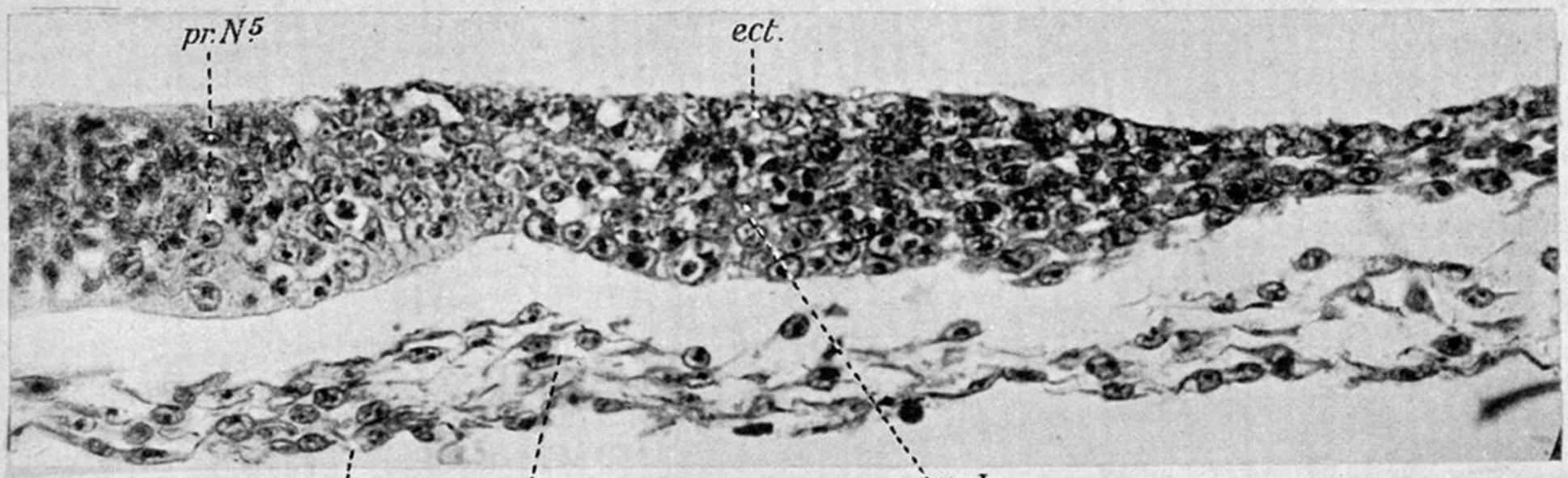

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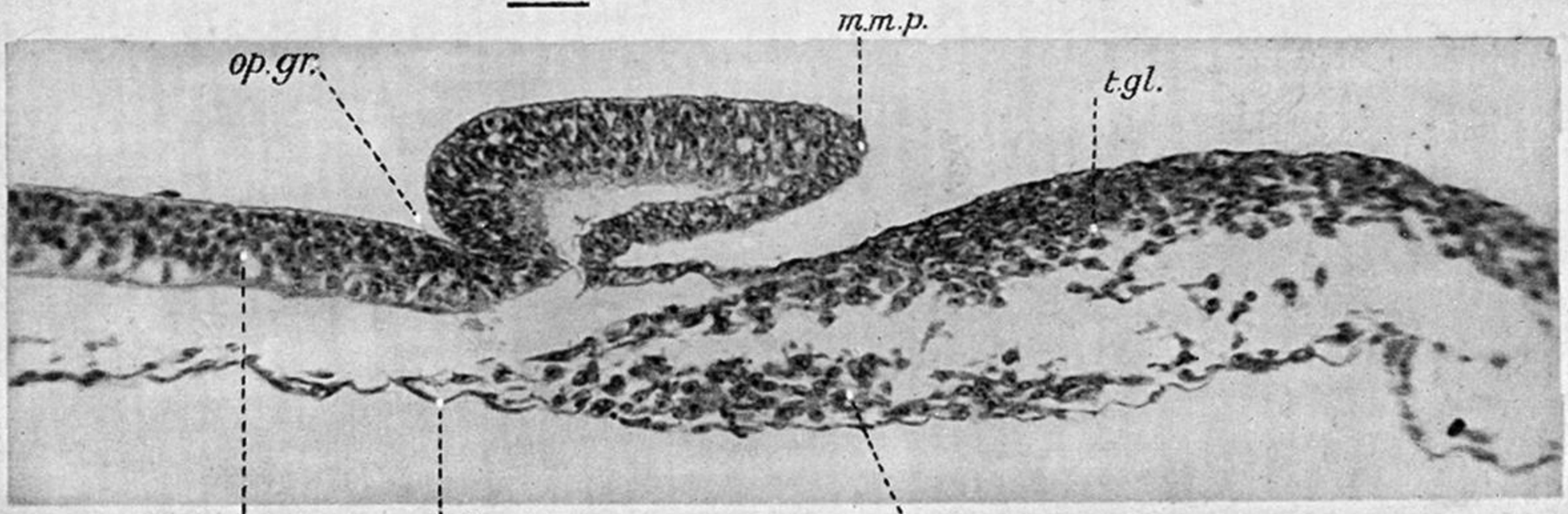

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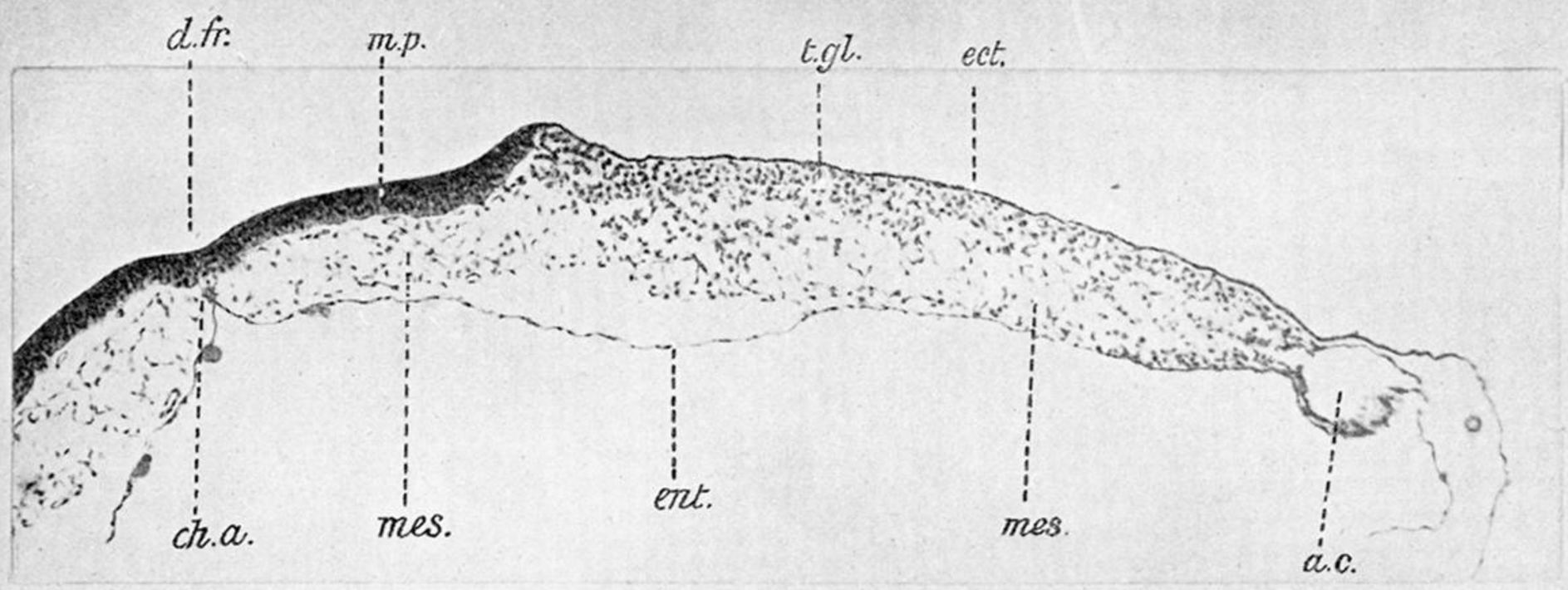
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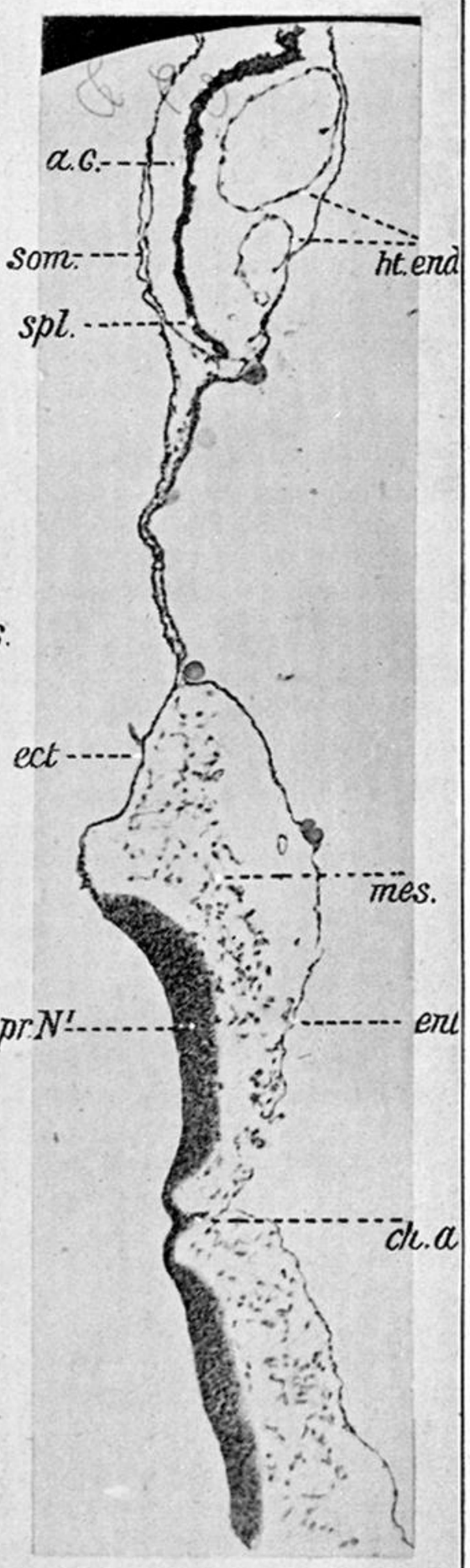
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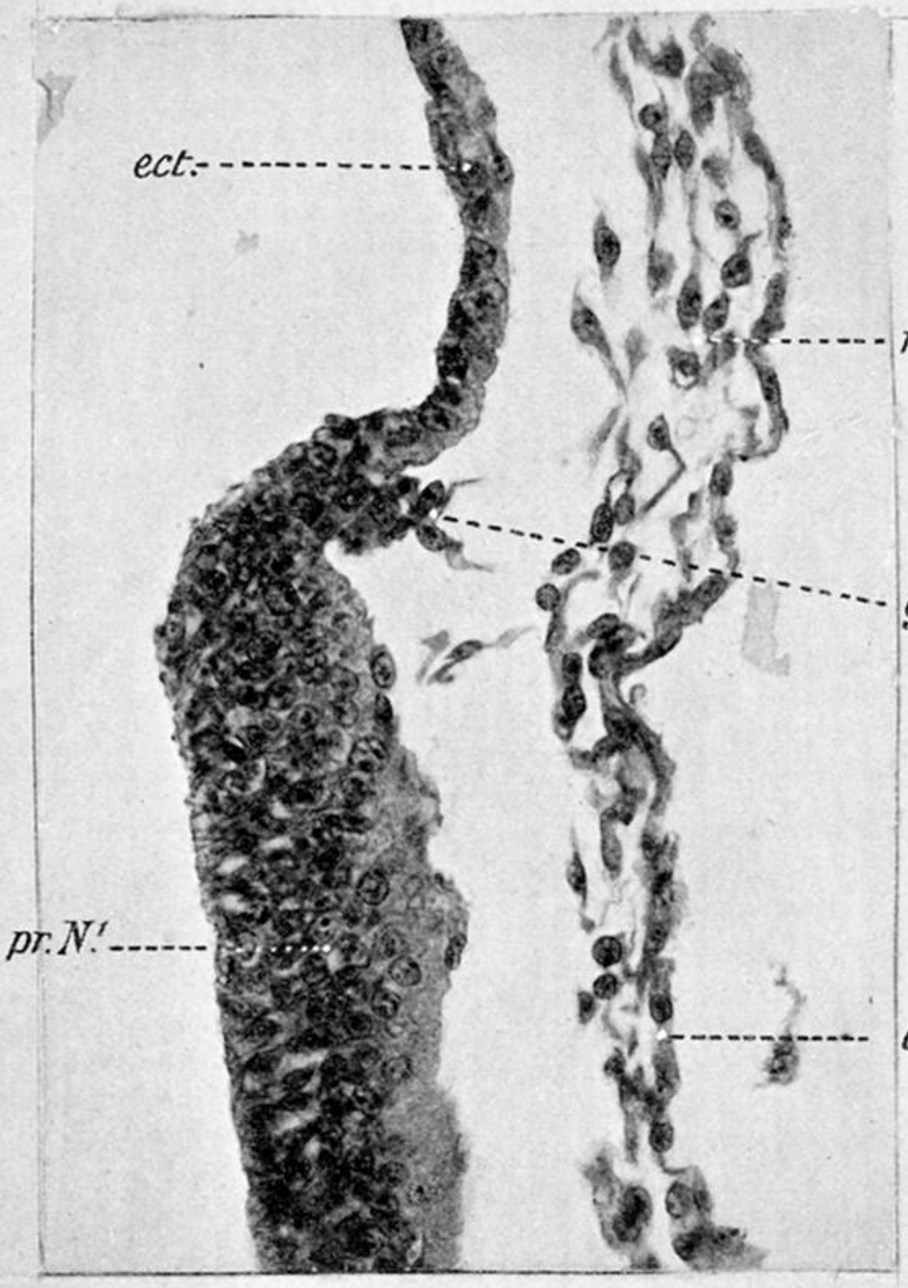
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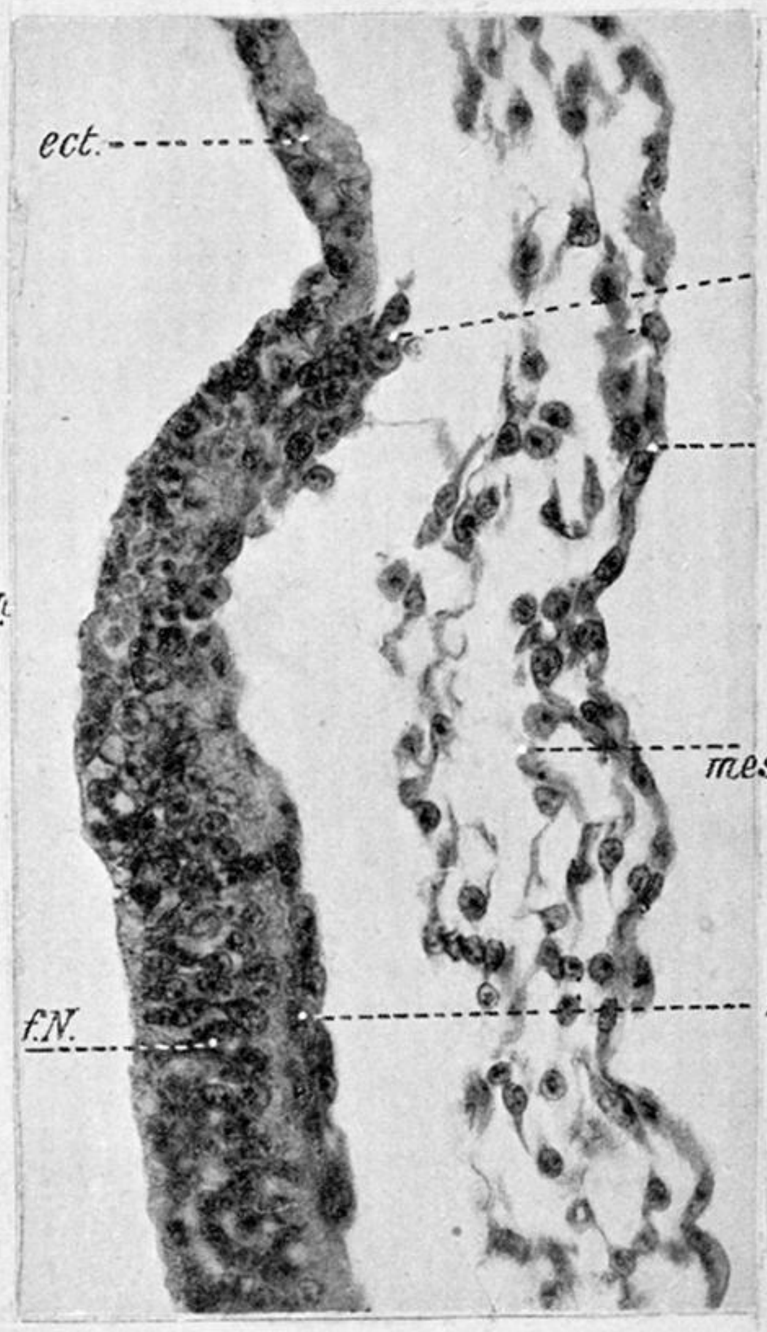
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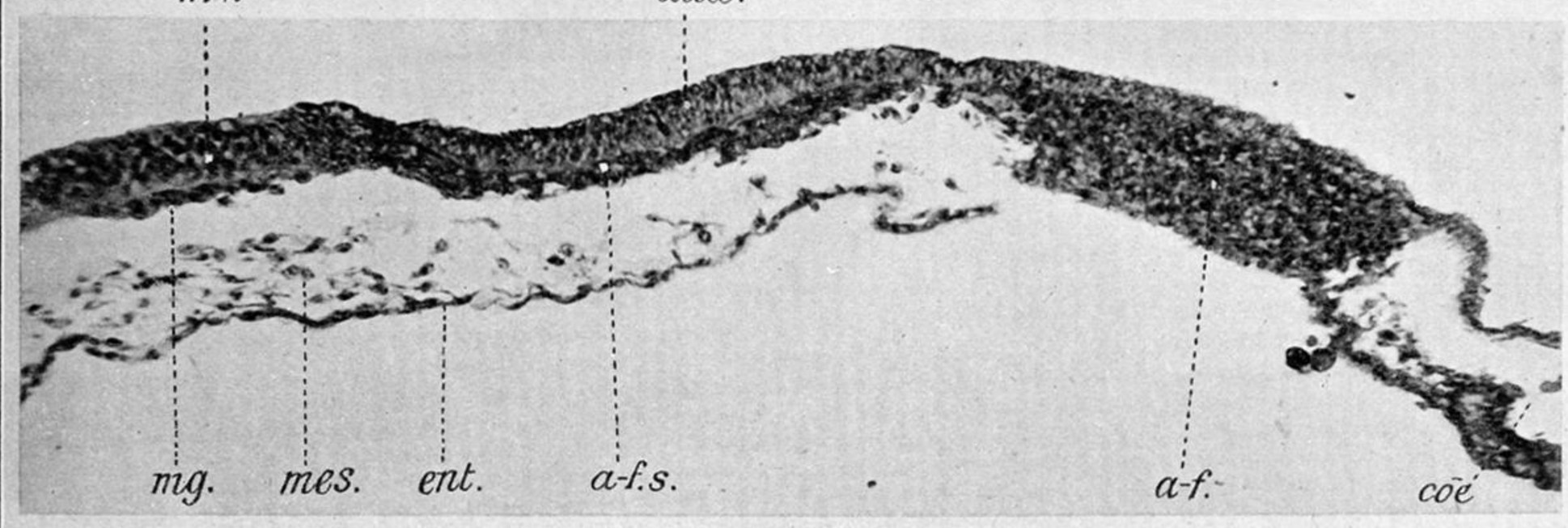

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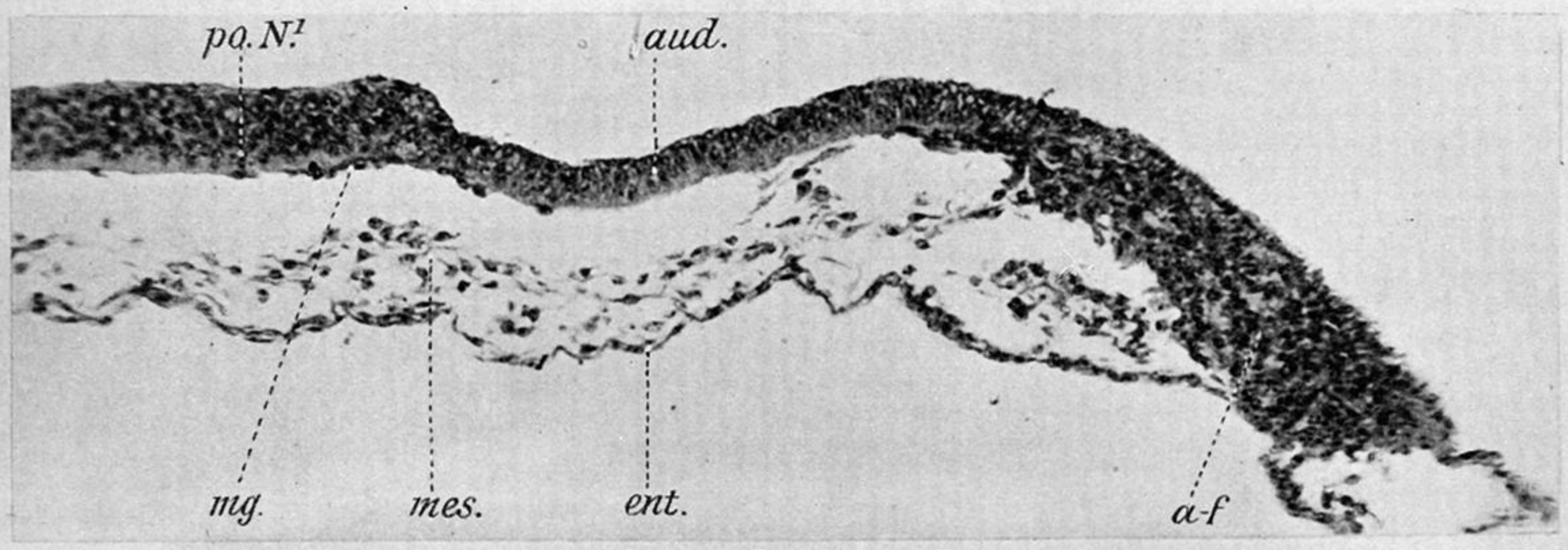

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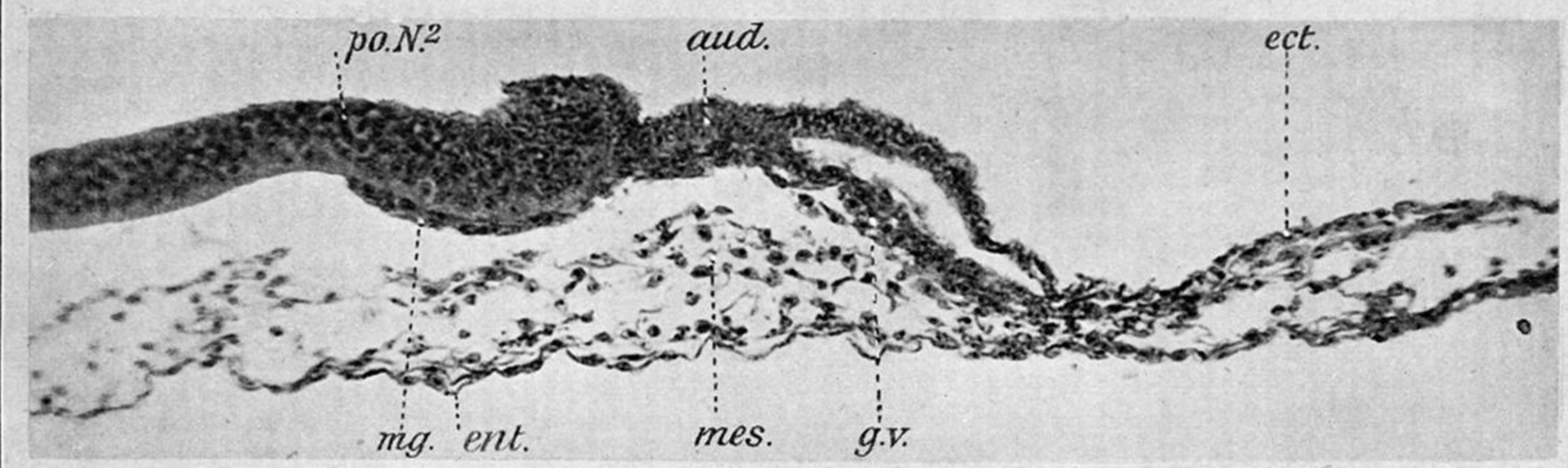

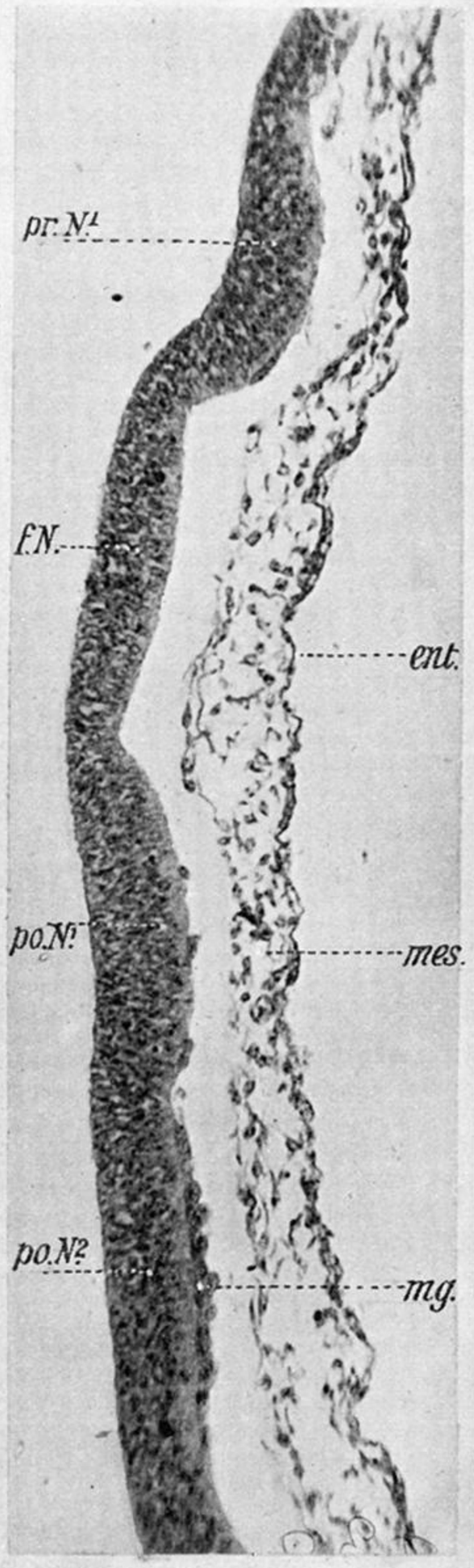

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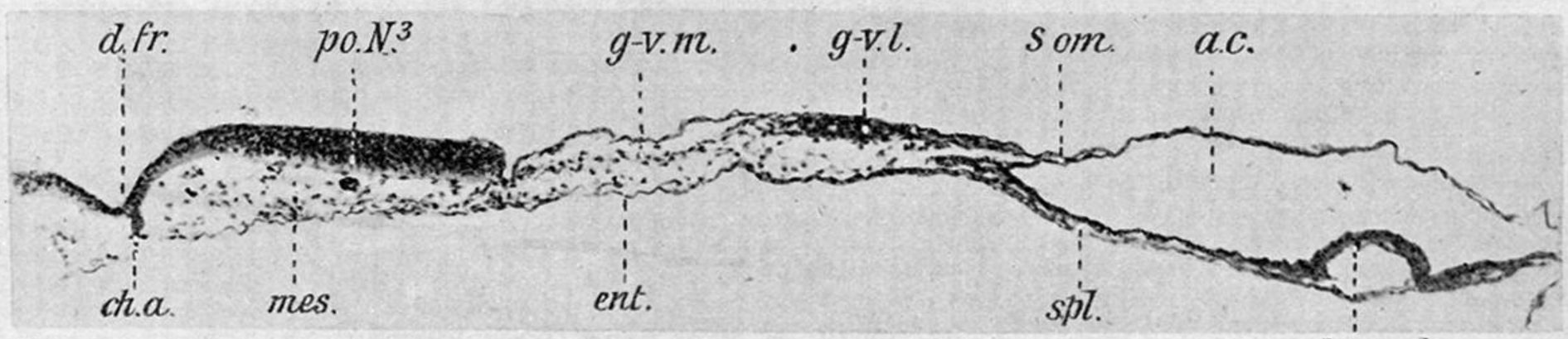

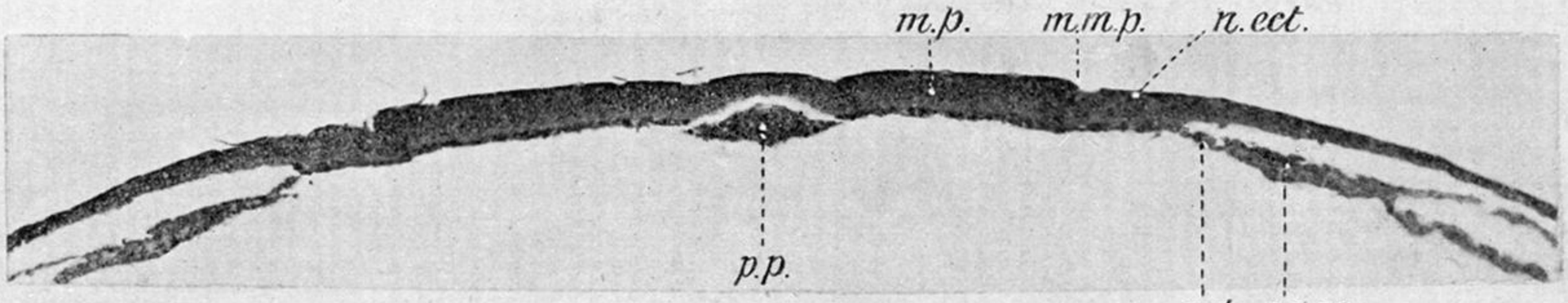

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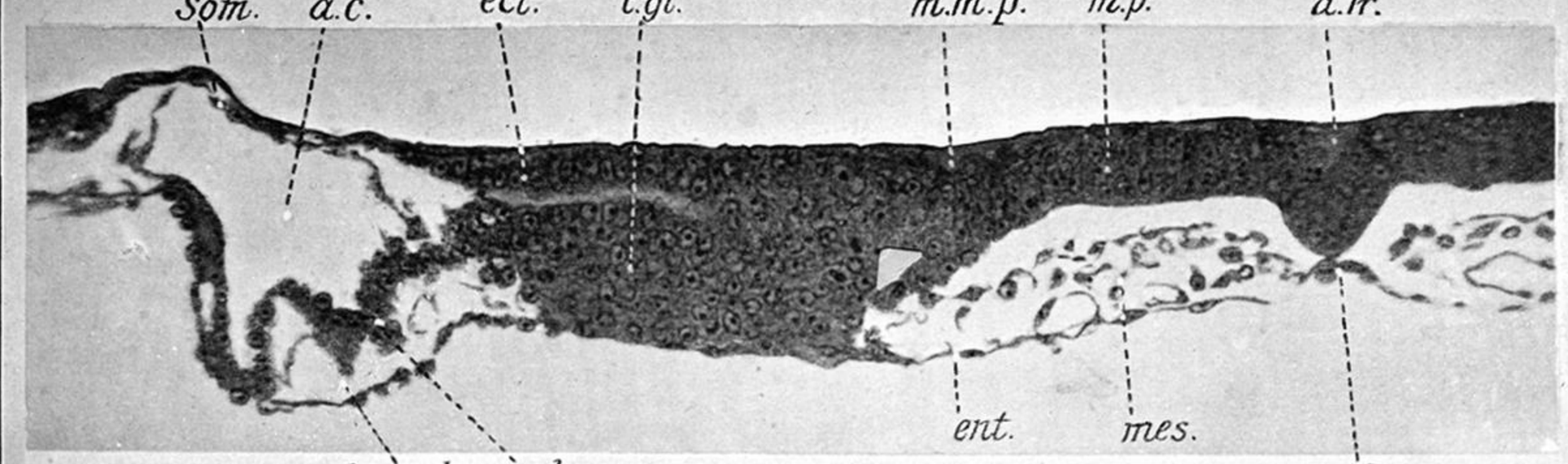

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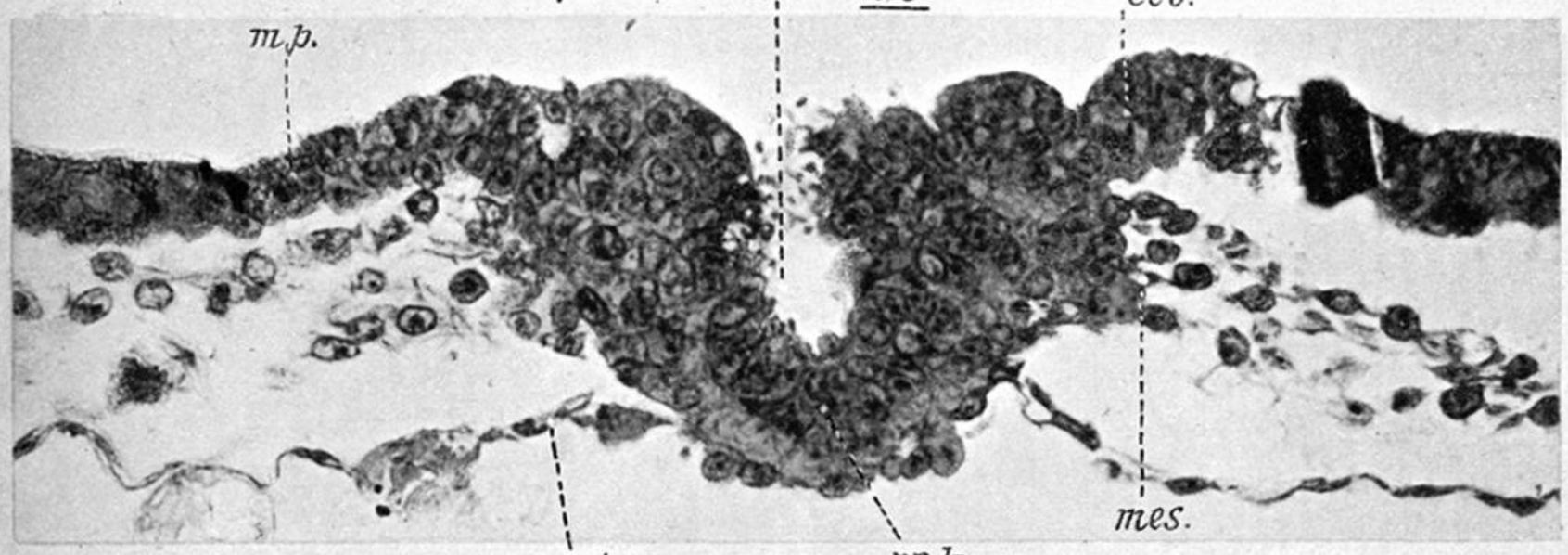

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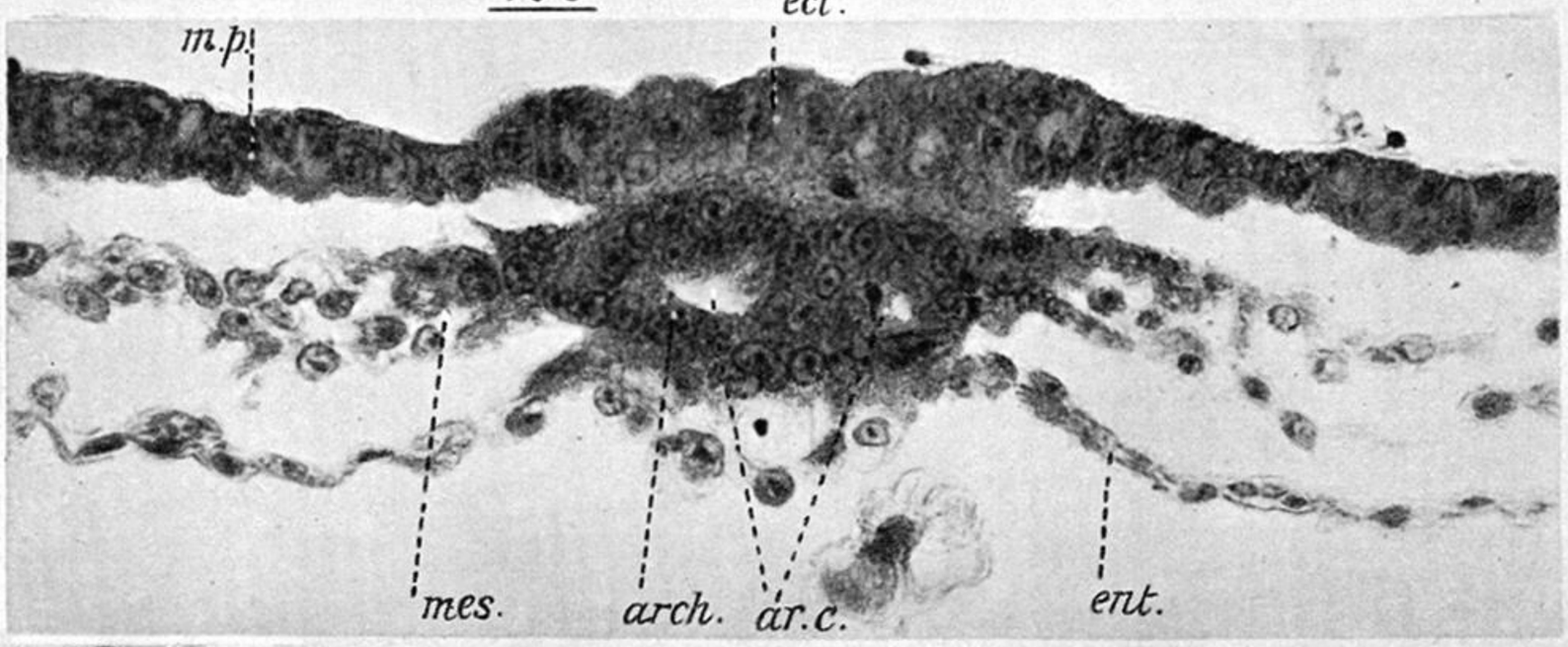

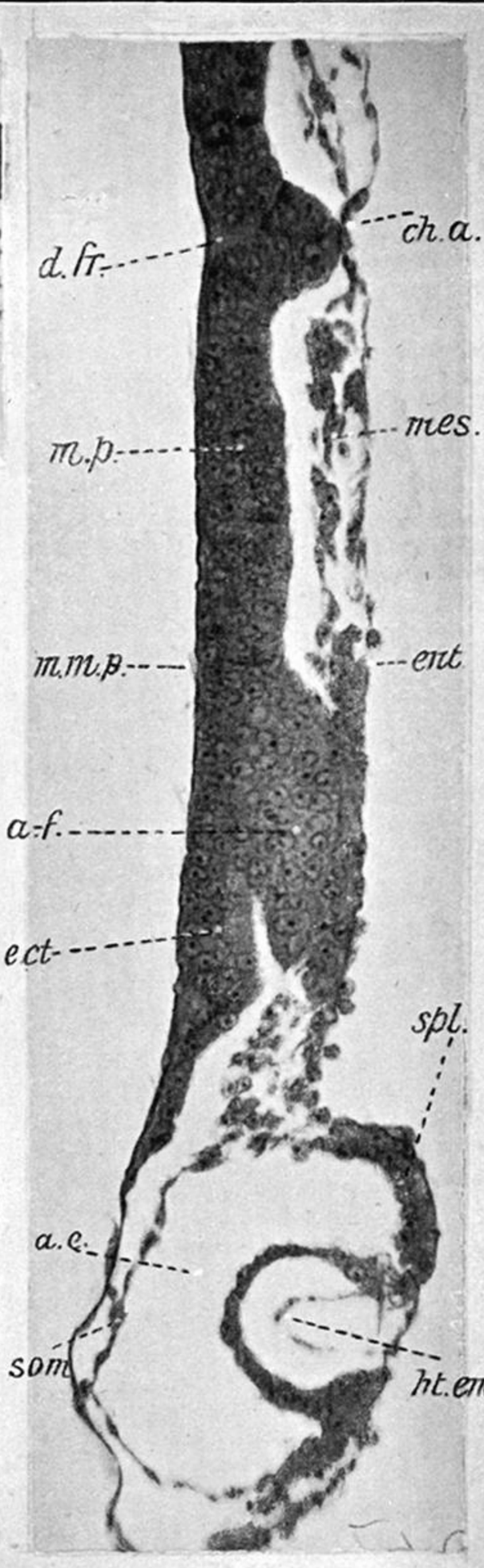

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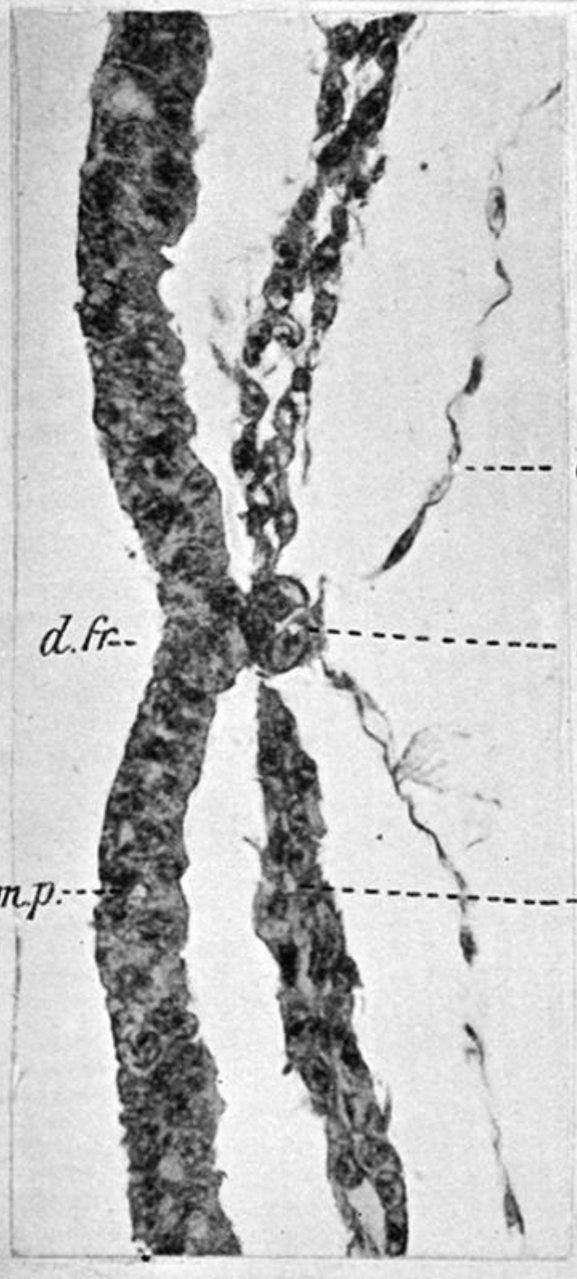

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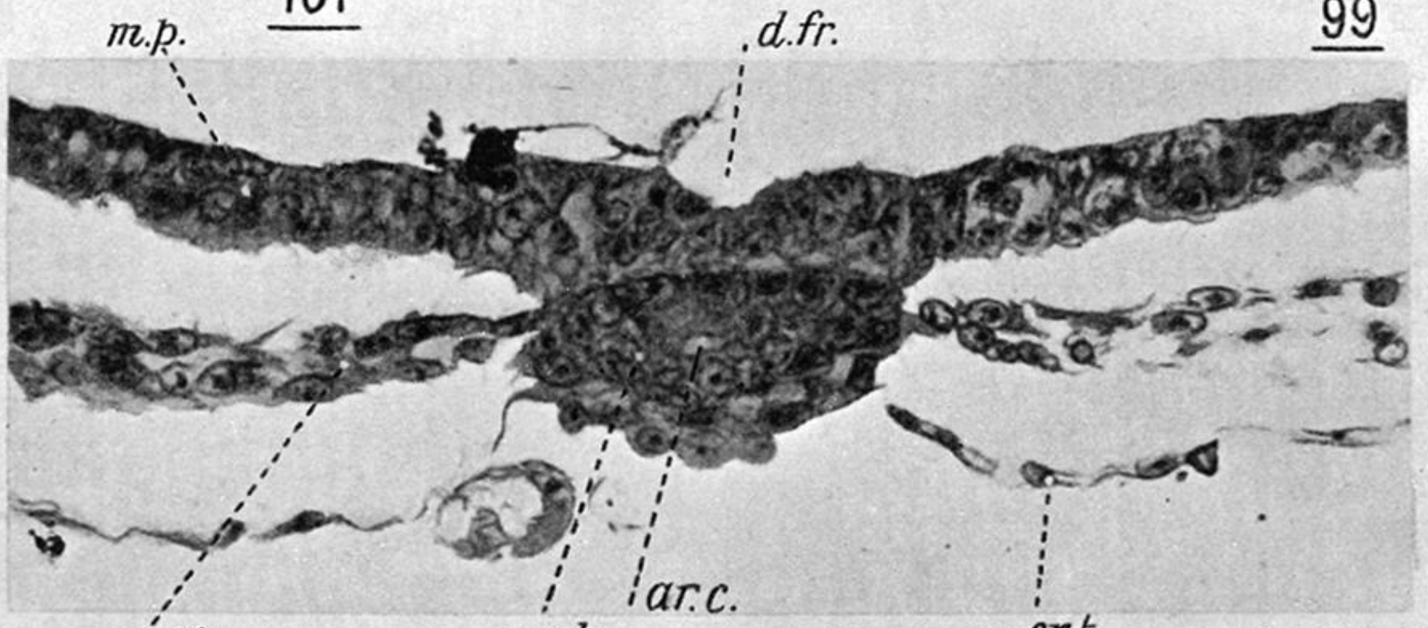

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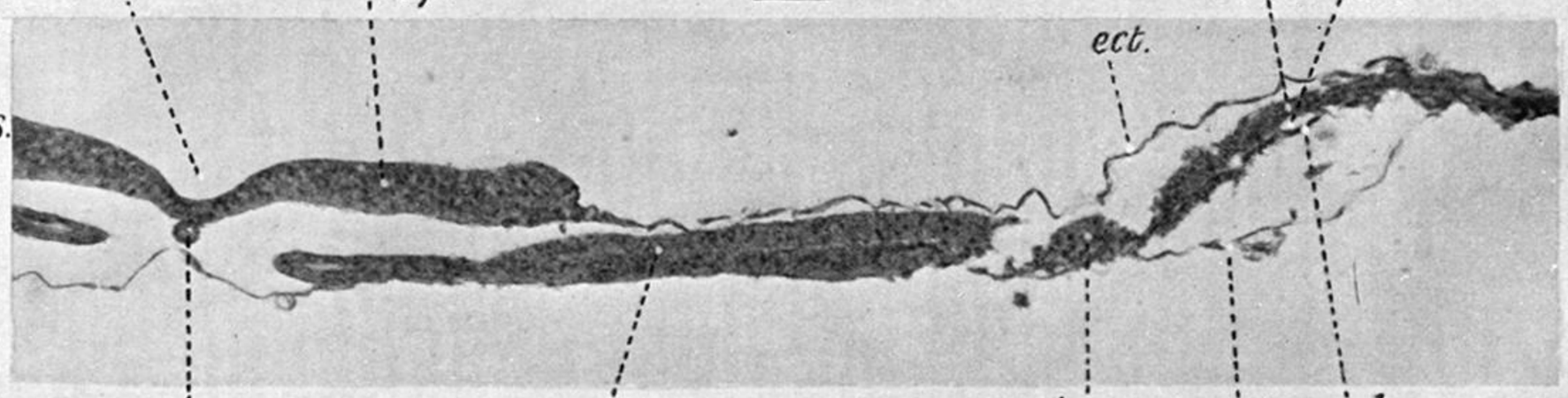

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